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RECOVERY FROM MYOSTATIC CONTRACTURE CAUSED BY TETANUS TOXIN*

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Tetanus toxin when injected into one limb of a cat, rabbit or rat causes a shortening of the extensor muscles of that leg. The leg is held rigidly extended. In from five to seven days after the injection, if the dose has been an adequate one, the extensor muscles become set at this shortened length and fail to relax even after section of the motor nerves. The muscles are in a condition of myostatic contracture.[1] We have seen tetanic contracture in the quadriceps femoris of the cat persist for as many as five days after section of the femoral nerve, disappearing gradually as atrophy set in.[2] If the nerves have not been cut and if the dose has been just a little less than would produce generalized tetanus, the contracture may persist in rabbits for months. In the rat and particularly in the guinea-pig, in which the susceptibility is greater and the margin between the dose of toxin producing local tetanus and that causing a fatal general tetanus is less, it has been the experience in the laboratory that recovery from local tetanus quite regularly occurs within from four to eight weeks.

It might be inferred from this that a muscle in the myostatic contracture caused by tetanus toxin had not been irreparably damaged. But there is no evidence recorded to show that, in those animals that recover, the local tetanus had ever progressed to the stage of myostatic contracture. The experiments recorded in this paper were undertaken to determine the extent to which a muscle after it has once been set in contracture by the action of tetanus toxin may regain its normal length, structure and function.

* Submitted for publication, Jan. 15, 1929.

* From the Institute of Neurology, Northwestern University Medical School.

1. Ranson, S. W., and Morris, A. W.: J. Comp. Neurol. **42**:99, 1926. Ranson, S. W., and Sams, C. F.: J. Neurol. & Psychopath. **8**:304, 1928. Davenport, H. K.; Ranson, S. W., and Stevens, E.: Microscopic Changes of Muscle in Myostatic Contracture of Local Tetanus, Arch. Path., this issue, p. 978.

2. Ranson, S. W.: Local Tetanus; A Study of Muscle Tonus and Contracture, Arch. Neurol. & Psychiat. **20**:663 (Oct.) 1928.

METHOD

In order to be sure that myostatic contracture had developed, it was not sufficient to place the animal under deep anesthesia. Only if the muscle failed to relax after section of the motor nerve or after the death of the animal could one be sure that nerve impulses were not responsible for the maintenance of the shortened state. But these conditions excluded the possibility of studying the recovery of the muscle. To meet this difficulty, it was decided to work with pairs of rats. One of each pair, as control, was put to death at the height of contracture; the other was allowed to live for several weeks or months and then was killed in order that the muscles might be studied and the extent of recovery determined.

Young adult rats were used and were paired according to weight, the two members of a pair coming within 10 Gm. of being the same weight. The toxin used was of such a strength that 0.25 cc. constituted a suitable dose, and this amount was injected into the muscles on the back of the right thigh in each rat. Although the amount injected was greater than in the experiments of Davenport, Ranson and Stevens[1] the toxin was much weaker and the actual dose less, and the contractures produced were less pronounced. The dose was necessarily somewhat below that required to produce the most pronounced contracture because it was essential to keep the animals alive until recovery occurred.

A number of the rats died of tetanus and five others lost their identification tags. Seven pairs were carried through to the completion of the experiment. The data in regard to these are given in the accompanying table.

The five that lost their identification tags weeks or months after the injection were roughly paired according to their weight at the end of the experiment with five others that had been killed at the height of the contracture. The data obtained from this group agreed in all essentials with the data obtained from the better controlled experiments recorded in the table.

When the rigidity was at its height from seven to thirteen days after the injection, the rats were anesthetized deeply with ether and the amount of relaxation of the right hind leg was tested. One rat of each pair was allowed to recover from the anesthetic and the other was killed. Immediately after the death of the latter, the amount of relaxation of its right leg was again tested. Then the gastrocnemii of both hind legs were removed and fixed under a tension of 50 Gm. in a diluted solution of formaldehyde U. S. P. (1:10). After fixation, the weight and length of the muscles and the length of an easily identifiable bundle of parallel muscle fibers in each was determined, and histologic sections were prepared according to the methods described by Davenport, Ranson and Stevens.[1]

After recovery had occurred, the surviving members of the pairs were treated in a similar manner.

RESULTS

All the rats developed typical local tetanus in the leg into which injections were made and a contracture in the gastrocnemius, which failed to relax completely under deep ether anesthesia. For example, the pair listed last in the table was anesthetized thirteen days after the injection. In both rats, when under deep anesthesia, the right ankle could be flexed to a right angle. One rat was killed, and after death the ankle could still be flexed only to a right angle. Sixty-two days later (seventy-five days after the injection), the other rat had fully recovered and was using the right leg as well as the left. But when this rat was

placed under deep anesthesia, the ankle joint moved freely until it was almost completely flexed; then resistance was encountered. This persisted after the death of the animal and even after the section of the achilles tendon. It was therefore due to changes in the ankle joint.

As shown in the table, the right gastrocnemii of the rats killed in from seven to thirteen days after injection were consistently shorter than the left control muscles. The average length of the seven left gastrocnemii was 27.8 mm. and of the seven right gastrocnemii 24.2 mm., representing an average shortening of 3.6 mm. On the other hand, the rats killed from fifty-six to 110 days after the injection had right gastrocnemii of approximately the same length as the left. This shows that, with time and a return to normal function, the shortened muscles returned again to approximately normal length.

In the rats killed from seven to thirteen days after the injection, the individual muscle fibers selected for measurement were, on the average, 3 mm. shorter in the muscles on the injected side than in the control

Measurements of the Gastrocnemius Muscle of the White Rat in the Contracture of Local Tetanus and After Recovery

During Contracture					After Recovery				
	Length of Muscle, Mm.		Length of Fibers, Mm.			Length of Muscle, Mm.		Length of Fibers, Mm.	
Days After Injection	Normal	Injected	Normal	Injected	Days After Injection	Normal	Injected	Normal	Injected
7	26.0	23.8	10.0	6.8	66	28.5	27.5	10.9	7.7
7	27.2	23.0	10.6	6.7	86	29.6	31.1	11.2	9.0
9	30.2	25.2	10.5	6.7	53	26.0	25.5	9.4	9.0
10	27.6	24.1	9.1	5.2	59	25.2	25.9	9.0	7.9
11	27.8	22.9	9.0	4.7	61	31.0	28.8	10.0	7.1
12	26.9	24.8	8.0	7.0	110	27.0	27.0	9.2	7.8
13	29.0	26.0	9.1	8.5	75	27.4	27.2	9.9	7.8
Average	27.8	24.2	9.5	6.5	Average	27.8	27.6	9.9	8.0

muscles. In the rats killed from fifty-six to 110 days after the injection, the muscle fibers of the right gastrocnemii were, on the average, 1.9 mm. shorter than the same fibers in the control muscles of their left legs.

It is a little difficult to understand how, on recovery from contracture, the muscles could have returned so nearly to their normal length and still have the individual muscle fibers showing an appreciable amount of shortening. It could be explained on the assumption that during the three weeks or more when the muscle was under continuous tension, before the tetanus subsided, the thin intramuscular fibrous septums became slightly stretched.

When the five rats that lost their identification tags were paired with five others of approximately the same weight, the five pairs thus obtained gave essentially the same results as the seven included in the table.

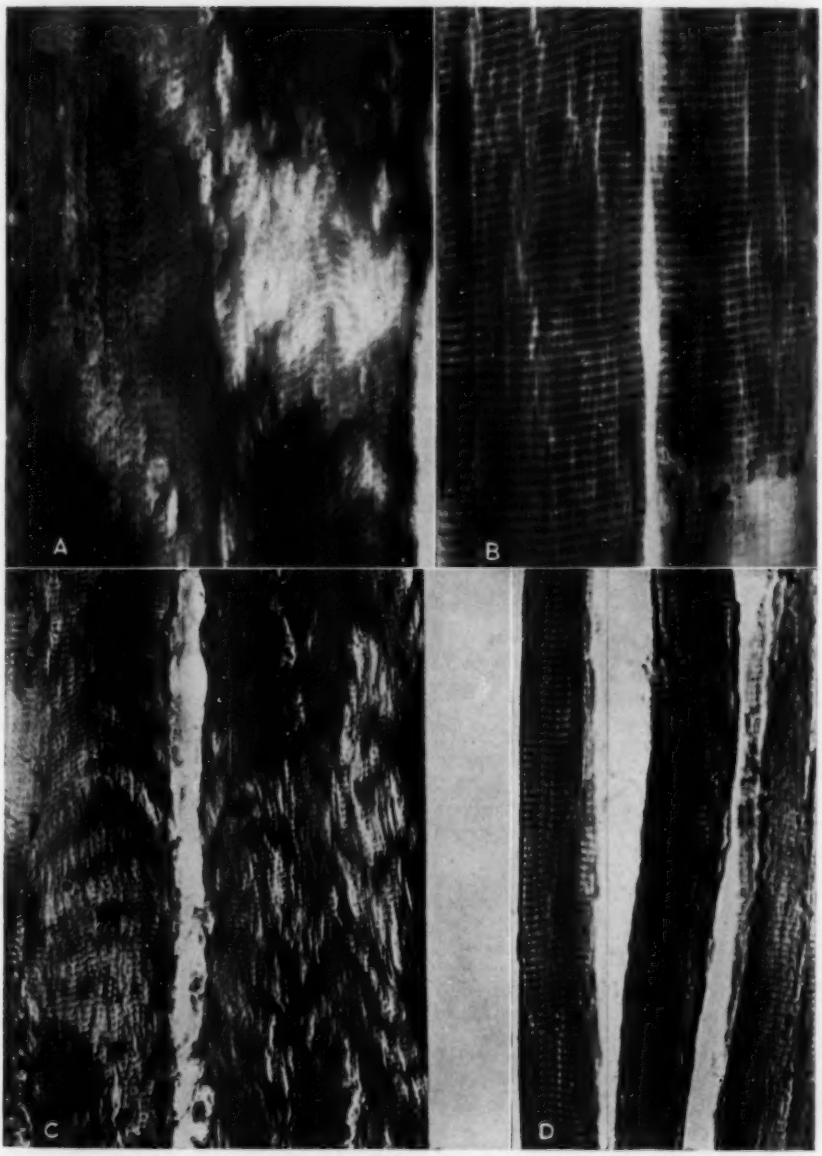
It is evident from these results that a muscle may recover from the myostatic contracture due to tetanus toxin so that the limb of which it forms a part can be used in a normal manner. The length of the muscle also returns approximately to normal, but some shortening of the individual muscle fibers remains.

The weight of the muscle was not altered by tetanic contracture in the animals killed from seven to thirteen days after the injection. But, in the animals that had recovered from the contracture, there was some slight atrophy of the muscle on the side which received the injection. This weighed, on the average, 0.96 Gm., as compared with 1.13 Gm. for the control muscle.

Histologic sections of the muscles were made and stained in the manner described by Davenport, Ranson and Stevens, except that all the material had been fixed in formaldehyde. The muscles that had been fixed at the height of contracture, from seven to thirteen days after the injection, showed the blurring of cross striations described by Davenport, Ranson and Stevens.[1] The individual myofibrils were more evident than in normal muscle, but were less tightly bound together and more tortuous (figs. *A* and *C*). Irregular patches of light staining, due to a decrease in the affinity of the anisotropic disks for the various dyes, also formed a conspicuous part of the picture. Nuclear proliferation was less marked than in the preparations described by Davenport, Ranson and Stevens, but a few tubes of sarcolemma, filled with nuclei and representing degenerating muscle fibers, were seen. In transverse sections, vacuoles could be seen in a few of the muscle fibers.

It is evident that the contractures produced in this investigation were not of such high grade as those investigated by Davenport, Ranson and Stevens.[1] The histologic changes were not as advanced, and there was less shortening of the muscles. Their results could not be duplicated in this investigation because the larger doses of toxin that they employed would, after two or three weeks, have caused the death of the animal, thus preventing the study of recovery. Nevertheless, the histologic changes, as well as the shortening, showed that the muscles were in myostatic contracture.

The muscles, which in from fifty-six to 110 days after the injection had recovered from the contracture and returned to approximately normal length, were of normal histologic appearance. The cross striations were regular and well defined (figs. *B* and *D*). The myofibrils were no longer abnormally prominent and the irregular, patchy staining had disappeared. The nuclei were normal in number and arrangement and the long masses of nuclei, representing degenerating fibers, were not seen. It is not possible to say whether these muscle fibers had regenerated or been replaced by connective tissue. It was not possible to see that there was any increase in connective tissue, and if any increase had occurred, it must have been slight.



A, a longitudinal section of the gastrocnemius muscle in myostatic contracture caused by tetanus toxin; × 570. *B*, a longitudinal section of the gastrocnemius muscle after recovery from myostatic contracture; × 570. *C*, a longitudinal section of the gastrocnemius muscle in myostatic contracture caused by tetanus toxin; × 400. *A*, a longitudinal section of the gastrocnemius muscle after recovery from myostatic contracture; × 400.

CONCLUSION AND SUMMARY

The gastrocnemius muscle of the white rat, which has been set at a shortened length in the myostatic contracture of local tetanus, may after from eight to twelve weeks recover approximately its normal length and be used again normally in locomotion. The muscle acquires again a normal histologic appearance. The muscle fibers show regular and well defined cross striations and nuclei of the normal number and distribution. There is no obvious increase in connective tissue.

A MORPHOLOGIC STUDY OF REGENERATION OF THE LIVER AFTER PARTIAL REMOVAL*

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The real meaning of "regeneration" is restoration of tissue or of a part of an organ at the site of its removal, and the term implies compensation, either structural, as of a part, or physiologic, as of a function. In the restoration of hepatic tissue after partial hepatectomy, the replacement does not occur at the stump from which the lobes were removed, but within the remaining lobes, so that "restoration" or "restitution" might describe the process more accurately. This process, however, has been known as "regeneration" in the medical literature, and so it would be confusing to offer a different title.

The idea of regeneration of the liver comes down from antiquity, first revealing itself in the myth concerning Prometheus, whose liver was gnawed on daily by the tormenting vulture at the behest of Jove. The first scientific observers to suggest the possibility of regeneration of the liver were Cruveilhier [1] and Andral.[2] Since then, this conception has been widely accepted but with considerable divergence of opinion as to the manner and the extent of the process. Albers [3] and Weismann [4] were unconvinced of any regenerative capacity, and Aschoff [5] stated that the reformation of any organ was never marked, least of all that of a glandular organ.

The literature on the regeneration of the liver is exceedingly comprehensive, surveying the process thoroughly from the aspects of both clinical and experimental pathology. The following men have contributed relatively complete reviews of it: von Podwyssozki,[6] von

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* Submitted for publication, Feb. 12, 1929.

* From the Division of Experimental Surgery and Pathology, The Mayo Foundation.

1. Cruveilhier, J., quoted by Milne, L. S.: The Histology of Liver Tissue Regeneration, J. Path. & Bact. **13**:127, 1909.

2. Andral, quoted by Melchior, Eduard: Ein Beitrage zur alkohlischen hypertrophischen Cirrhose (Hanot-Gilbert) mit besonderer Berücksichtigung der Regenerationsvorgänge des Leberparenchyms, Beitr. z. path. Anat. u. z. allg. Path. **42**: 479, 1907.

3. Albers, quoted by Melchior (footnote 2).

4. Weismann, A., quoted by Milne (footnote 1).

5. Aschoff, L.: Regeneration und Hypertrophie, Ergebn. d. allg. Path. u. path. Anat. **5**:22, 1898.

6. Von Podwyssozki, W., Jr.: Experimentelle Untersuchungen über die Regeneration der Drüsengewebe, Beitr. z. path. Anat. u. z. allg. Path. **1**:259, 1886.

Meister,[7] Meder,[8] Barbacci,[9] MacCallum,[10] Melchior,[11] Muir,[12] Milne,[13] Hess,[14] Herxheimer and Garlach [15] and Schultz, Hall and Baker.[16]

CLINICAL PATHOLOGY

Regeneration of the liver has been studied whenever any rapid, widespread destructive lesion of this organ has occurred. The favored lesions have been acute and subacute yellow atrophy because of the speed and vigor with which in these conditions destruction of hepatic cells takes place.

Marchand [17] was the first accurately to describe acute yellow atrophy and the subsequent regeneration from the injured hepatic cells. These, he said, gave rise to new cells. The apparent proliferation of the ends of small bile ducts was due to either degeneration or regeneration and rearrangement of the injured parenchymal cells. He stated definitely that the bile ducts did not proliferate, and did not form new hepatic cells. His description of acute yellow atrophy, however, remains as authoritative as when he wrote it.

Meder,[8] confirming Marchand's work, pointed out that the evidence of proliferation of the bile ducts was most marked when the destruction

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7. Von Meister, Valerian: Recreation des Lebergewebes nach Abtragung ganzer Leberlappen, Beitr. z. path. Anat. u. z. allg. Path. **15**:1, 1894.

8. Meder, E.: Ueber acute Leberatrophie mit besonderer Berücksichtigung der dabei beobachteten Regenerationserscheinungen, Beitr. z. path. Anat. u. z. allg. Path. **17**:143, 1895.

9. Barbacci, O.: Ueber Ausgang der acuten Leberatrophie in multiple, knotige Hyperplasie, Beitr. z. path. Anat. u. z. allg. Path. **30**:49, 1901.

10. MacCallum, W. G.: Regenerative Changes in the Liver After Acute Yellow Atrophy, Johns Hopkins Hosp. Rep. **10**:375, 1902; Regenerative Changes in Cirrhosis of the Liver, J. A. M. A. **43**:649 (Sept. 3) 1904.

11. Melchior, Eduard: Ein Beitrage zur alkoholischen hypertrophischen Cirrhose (Hanot-Gilbert) mit besonderer Berücksichtigung der Regenerationsvorgänge des Leberparenchyms, Beitr. z. path. Anat. u. z. allg. Path. **42**:479, 1907.

12. Muir, Robert: On Proliferation of the Cells of the Liver, J. Path. & Bact. **12**:287, 1908.

13. Milne, L. S.: The Histology of Liver Tissue Regeneration, J. Path. & Bact. **13**:127, 1909.

14. Hess, Otto: Ueber die bei akuten gelben Leberatrophie auftretenden Regenerationsprozesse, Beitr. z. path. Anat. u. z. allg. Path. **56**:22, 1914.

15. Herxheimer, Gotthold, and Garlach, Werner: Ueber Leberatrophie und ihr Verhältnis zu Syphilis und Salvarsan, zugleich ein Beitrag zur Frage der Leberzellregeneration, Beitr. z. path. Anat. u. z. allg. Path. **68**:93, 1921.

16. Schultz, E. W.; Hall, E. M., and Baker, H. V.: Repair of the Liver Following the Injection of Chloroform into the Portal Vein, J. M. Research **44**: 207, 1923.

17. Marchand, F.: Ueber Ausgang der acuten Leberatrophie in multiple knotige Hyperplasie, Beitr. z. path. Anat. u. z. allg. Path. **17**:206, 1895.

of the hepatic cells was most severe. The budding at the end of the bile ducts was true proliferation, and not degeneration of hepatic cells, as Marchand had believed. In one of his five cases, Meder found mitotic figures in the buds of the bile duct continuous with new hepatic cells. He concluded that when the destruction of the tissue of the liver had been severe, the bile ducts participated in the formation of new hepatic cells.

Ribbert [18] agreed with Marchand that the bile ducts did not proliferate or form new hepatic cells, and that the regeneration was from remaining cell strands. Carraro [19] believed that there was proliferation of the bile ducts, but that these duct beds never became hepatic cells. Stroebe [20] maintained that if a sufficient amount of hepatic tissue were destroyed, the interlobular bile ducts would sprout and form new hepatic cells. Barbacci [9] agreed with Stroebe, believing that if injured hepatic cells remained, they divided, but that otherwise the embryonic manner of regeneration prevailed, namely, that the interlobular epithelium of the bile duct proliferated and formed new hepatic cells.

MacCallum [10] (1902) found mitotic figures in the tips of proliferating bile ducts. The presence of bile in the drainage capillaries indicated functional activity of the new hepatic cells. He noted the distortion of the new lobules. And finally, if all the parenchymal cells were destroyed, the bile duct cells would multiply and differentiate, adopting the characteristics of hepatic cells. On the other hand, if any of the hepatic cells remained, they gave rise to new hepatic cells and the bile ducts took little or no part in the process.

Milne [13] offered another interpretation of the bile duct buds. They were the hardy, persistent interlobular canaliculi which became embedded in the new connective tissue, and served to connect the remaining or regenerating hepatic cells with larger bile ducts. Rarely, these ducts might show a meager, local proliferation along their course, but new hepatic cells did not arise from them. Hepatic tissue was restored by direct division of the remaining hepatic cells.

Later, Hess [14] reviewed this aspect of regeneration in acute yellow atrophy and agreed with MacCallum, except that he believed the epithelium of the bile duct and injured hepatic cells was equivalent for purposes of regeneration. More recently, Herxheimer and Garlach [15] have shown this with fine serial sections.

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18. Ribbert, quoted by Hess (footnote 14) and by MacCallum (footnote 10).

19. Carraro, quoted by Hess (footnote 14).

20. Stroebe, H.: Zur Kenntnis der sogenannten acuten Leberatrophie, ihrer Histogenese und Aetiologie mit besonderer Berücksichtigung der Spätstadien, Beitr. z. path. Anat. u. z. allg. Path. **21**:379, 1897.

In the different types of atrophy of the liver there are many hyperplastic nodules which represent varying degrees of regeneration, dependent on the duration of the atrophy. M'Donald and Milne [21] maintained that the hyperplastic, nodular foci arose only from the uninjured hepatic cells and not from the bile ducts. Miller and Rutherford [22] reported sixteen cases of atrophy of the liver in which the budding at the ends of the bile duct, with enlargement of nuclei and frequent mitosis, was the first evidence of regeneration.

Hess [23] observed new hepatic cells regenerating from the epithelium of the bile duct in three cases of rupture of the liver, and contrasted it with the embryonic process. He did not see any mitotic figures, but his cases were of several days' duration. Muir [12] described three similar cases, which showed ductlike structures growing from the ends of trabeculae. He believed this proliferation to be an attempt of the hepatic cells to repair a breach in the continuity of the duct system.

Regeneration of the liver has been observed in cirrhosis and carcinoma of the liver.[24] The question of carcinoma arising from the hyperplastic nodules in cases of cirrhosis has long been discussed. Many primary hepatic neoplasms are associated with cirrhosis.

MacCallum [10] (1904) noted the similarity of regeneration in cirrhosis and subacute yellow atrophy. The remaining hepatic cells divided by mitosis and the bile ducts penetrated the scar tissue, sending forth buds, which became hepatic cells.[25] The epithelium of the bile duct was more resistant than the hepatic cell, but both types of cells participated equally in regeneration.

So-called compensatory regeneration of the liver has also been noted in suppurative hydatid cysts, echinococcus cysts, syphilitic obliteration of vessels to one lobe and in chronic venous congestion.[13]

Mention should be made of the opinions on division of hepatic cells. Prior to the recognition of karyokinesis, these cells were supposed to divide by amitotic, or direct, division. Tillmanns [26] observed mitotic figures in hepatic cells near scars, interpreting them as evidence of

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21. M'Donald, Stuart, and Milne, L. S.: Subacute Liver Atrophy, J. Path. & Bact. **13**:161, 1909.

22. Miller, James, and Rutherford, Andrew: Liver Atrophy, Quart. J. Med. **17**:81, 1923.

23. Hess, Karl: Beiträg zur Lehre von den traumatischen Leberrupturen, Virchows Arch. f. path. Anat. **121**:154, 1890.

24. Milne, L. S.: Primary Epithelial Tumour Growth in the Liver, J. Path. & Bact. **13**:348, 1909.

25. MacCallum, W. G.: Textbook of Pathology, Philadelphia, W. B. Saunders Company, 1917, p. 305.

26. Tillmanns, H.: Experimentelle und anatomische Untersuchungen über Wunden der Leber und Niere. Ein Beitrag zur Lehre von der antiseptischen Wundheilung, Virchows Arch. f. path. Anat. **78**:437, 1879.

regeneration. Ziegler and Obolonsky [27] noted mitosis after phosphorus poisoning. Bizzozero and Vassale [28] observed mitotic figures in the livers of animals, and regarded them as evidence of physiologic replacement.

Balbiani and Henneguy [29] described amitotic and mitotic division of cells in the same specimen. Adler [30] noted both amitosis and mitosis in cases of phosphorus poisoning and eclampsia. Von Podwyssozki [6] and Ponfick [31] described mitosis in regenerating liver. Milne [13] and Miller and Rutherford [22] believed that the division was chiefly by amitosis. Whipple and Sperry [32] found mitotic figures after prolonged chloroform anesthesia, usually most numerous on the second day. Schultz, Hall and Baker [16] observed only a few mitotic figures, but believed this to be the prevalent mode of division because of lack of evidence of direct division. Maximow [33] found hepatic cells dividing by mitosis under conditions of tissue culture.

EXPERIMENTAL PATHOLOGY

Experiments in this field were first conducted in Italy. Colucci [34] injured the continuity of the liver in guinea-pigs and noted new hepatic cells, which he believed arose from leukocytes by nuclear division. Corona [35] and Tizzoni [36] observed the proliferation of hepatic cells and believed it due to direct mechanical action causing division of the cells. Griffini [37] noted the transformation of cords of epithelial cells into small hepatic cells to form a new trabecular system. The epithelium to which

27. Ziegler, E., and Obolonsky, N.: Experimentelle Untersuchungen über die Wirkung des Arseniks und des Phosphors auf die Leber und die Nieren, Beitr. z. path. Anat. u. Physiol. **2**:291, 1888.

28. Bizzozero, G., and Vassale, G.: Ueber die Erzengung und die physiologische Regeneration der Drüsenzellen bei den Säugethieren, Virchows Arch. f. path. Anat. **110**:155, 1887.

29. Balbiani and Henneguy, quoted by Milne (footnote 13).

30. Adler, L.: Ueber helle Zellen in der menschlichen Leber, Beitr. z. path. Anat. u. z. allg. Path. **35**:127 1904.

31. Ponfick, E.: Experimentelle Beiträge zur Pathologie der Leber, Virchows Arch. f. path. Anat., 1895, vol. 138, suppl., p. 81.

32. Whipple, G. H., and Sperry, J. A.: Chloroform poisoning. Liver Necrosis and Repair, Bull. Johns Hopkins Hosp. **20**:278, 1909.

33. Maximow, A. A.: Some Applications of the Method of Tissue Culture to the Solution of Pathological Problems. Mayo Foundation Lectures (unpublished data).

34. Colucci, V., quoted by von Podwyssozki, Jr. (footnote 6).

35. Corona, quoted by von Podwyssozki, Jr. (footnote 6).

36. Tizzoni, quoted by von Podwyssozki, Jr. (footnote 6).

37. Griffini, quoted by von Podwyssozki, Jr. (footnote 6) and Melchior (footnote 11).

he referred was probably that lining bile ducts. Canalis [38] removed small blocks from the livers of dogs and guinea-pigs, and saw the proliferation of hepatic cells and a new formation of bile ducts, which, however, he did not believe arose from the bile ducts. He did not observe the transition of bile ducts to hepatic cells, as Griffini had suggested.

Von Podwyssozki [6] removed small wedges from the livers of rats, cats, guinea-pigs and rabbits. His work was the first carried on in accord with modern standards. Evidence of regeneration in adjacent hepatic cells appeared within twenty-four hours. In rats, mitotic figures appeared near the wound within two and a half days. Bile ducts began to sprout into the scar tissue by the fourth day, and by the tenth had become hepatic cells. Regeneration was predominantly from the bile ducts in guinea-pigs and rabbits, and from the hepatic cells in cats and rats. Prus [39] confirmed these results the following year.

Ponfick [40] removed the liver partially from rabbits and dogs, removing three or even four of the major lobes, an equivalent of 75 per cent. A three-fold increase of hepatic tissue occurred, which he attributed to functional stimulation arising from physiologic lack. The newly formed tissue functioned normally because it arose from an entirely normal substratum, in contrast with the scarred soil in which hepatic cells arose after cirrhosis. After congestion, extreme hypertrophy occurred which reached the optimum within a few weeks. The new hepatic cells were swollen and were more voluminous with pale, bright cytoplasm, and their nuclei were somewhat larger than normal. Mitosis occurred by the second day. Nuclear division frequently preceded that of the cytoplasm, which lagged behind a definite interval. The regenerating field was not uniform, being speckled with pairs of new cells, which had blossomed forth as a stately procession of crops. Eventually, these new cells became so numerous that they formed islands, penetrating the original tissue in an harmonious though despotic sequence. The vascular system regenerated rapidly, but the radial arrangement of blood vessels was supplanted by one of the cavernous type.

The epithelium of the coarser bile passages proliferated, producing an unevenness, not unlike the ruffles of a collar, by the third day. Mitosis was noted in this proliferating epithelium, which was confined to the outlines of the original system. Ponfick did not observe growth by sprouts or off-shoots of the epithelium of the bile duct. The capillaries of the bile ducts regenerated less quickly than the hepatic cells. The narrow, uneven appearance of capillaries of the bile duct in the centers

38. Canalis, quoted by Hess (footnote 14) and by Melchior (footnote 11).

39. Prus, quoted by Hess (footnote 14).

40. Ponfick, E.: Ueber Leberestirpation, Jahresb. d. schles. Gesellsch. f. vaterl. Kult. **67**:75, 1889; Ueber Leberresection und Leberrecreation, Verhandl. d. deutsch. Gesellsch. f. Chir. **19**:28, 1890; also footnote 31.

of islands of regenerated glandular tissue he attributed to an overproduction of new gland cells or their too hasty connection with the old duct system. The ground plan remained unchanged, since regeneration occurred through interposition. The hypertrophy was built on the framework and foundation of old lobules and trabeculae. The lobules lost their former regularity and increased in all dimensions. Growth was slow at first for two days and then was accelerated, lasting three or four weeks. About the circumference of old lobules and centering always on branches of the hepatic veins were groups of new cells or excrescences which gave the lobule a cloverlike or heartlike shape.

Von Meister [7] corroborated Ponfick's observations. He asserted that although 75 per cent of the liver was removed, there would be restoration up to 80 per cent of the original weight, at least in rats, rabbits and dogs. Mann and Magath [41] also noted this in dogs. The process required from forty-five to sixty days and was more rapid in young, strong animals. Von Meister regarded it as compensatory hypertrophy of old lobules from hyperplasia of their cellular elements. Growth was subsequent to pressure outward of the increased number of cells. The old cells made way for the new and the lobules enlarged to two, three or even four times their former size. Proliferation, as indicated by mitosis, began on the second day in the peripheral zones. Cells near the centers of the lobules remained in a latent stage due to pressure from the strong growth at the periphery. The bile ducts and blood vessels did not take any special part in the process but were carried along by the hypertrophy. The remaining lobes were individual organs, each having the power of hypertrophy in case the other was extirpated.

Flöck [42] arrived at similar conclusions. Regeneration appeared first at the periphery of the lobules and the process was completed in forty-four days. The lobular hypertrophy was compensatory and dependent on the proliferation and hypertrophy of peripheral cells. Bile ducts and blood vessels played a secondary part, simply following in the footsteps of the regenerating hepatic cells.

Mall [43] pointed out that the so-called hypertrophied lobules were in reality new compound lobules. The old lobules sprouted and gave rise to new lobules of normal size. The apparent hypertrophy lay in the failure to recognize the newly formed compound lobules which had arisen from the periphery of the old ones. He observed that regeneration occurred chiefly at the periphery, but that this zone corresponded

41. Mann, F. C., and Magath, T. B.: The Production of Chronic Liver Insufficiency, Am. J. Physiol. **59**:485, 1922.

42. Flöck, quoted by Herxheimer and Garlach (footnote 15) and by Melchior (footnote 11).

43. Mall, F. P.: A Study of the Structural Unit of the Liver, Am. J. Anat. **5**:227, 1906.

to the center of the portal unit. Mitosis was rare and binucleate cells common. In the livers of human beings which showed regeneration, mitotic figures were found in the region of the terminal bile ducts. Schaper and Cohen [44] believed that when regeneration formed typical lobules, the growth had taken place within the more minute bile ducts.

Janson [45] ligated the hepatic artery to various lobes of the liver in rabbits and found regeneration of those lobes with proliferation of the bile ducts within. These he called "pseudo bile ducts" and regarded them as atrophic cells of the liver. He believed that in the case of extreme regeneration, the bile ducts might give rise to hepatic cells, but he did not note such a phenomenon.

Rous and Larimore [46] ligated branches of the portal vein to certain lobes in rabbits and dogs and found atrophy of those lobes with hypertrophy of the others the portal circulation of which was uninjured. The atrophy was simple; that is, there was no degeneration or increase of connective tissue. Also, the atrophy was conditional, since it did not progress when hypertrophy was checked in the other lobes by ligation of their drainage bile ducts.

De Bary,[47] working on dogs, excised portions of the liver and noted the metamorphosis of the trabeculae of the cells occurring in small, dark, ductlike structures. Porcile [48] injected turpentine into the livers of rabbits and noted a proliferation of the bile ducts and a marked similarity of the new cells of the bile duct to the cells of the liver. Hayami [49] injected a 10 per cent solution of aleuronat in an emulsion of 6 per cent sodium chloride and found evidence of regeneration in four days. The regenerating hepatic cells he regarded as arising from sprouts from the bile ducts.

Milne [13] tried, among several methods, a method of partial hepatectomy which consisted in the ligation of branches of the portal vein and hepatic artery passing to certain lobes, waiting three weeks for atrophy and fibrosis, and then removing the shrunken, fibrotic lobes with less danger of hemorrhage. Regeneration began on the third day

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44. Schaper and Cohen, quoted by Hess (footnote 14) and by Milne (footnote 13).

45. Janson, Carl: Ueber Leberveränderungen nach Unterbindung der Arteria hepatica, Beitr. z. path. Anat. u. z. allg. Path. **17**:505, 1895.

46. Rous, Peyton, and Larimore, Louise D.: Relation of the Portal Blood to Liver Maintenance; a Demonstration of Liver Atrophy Condition on Compensation, J. Exper. Med. **31**:609, 1920.

47. De Bary, quoted by Hess (footnote 14) and by Milne (footnote 13).

48. Porcile, Vittorio: Untersuchungen über die Herkunft der Plasmazellen in der Leber, Beitr. z. path. Anat. u. z. allg. Path. **36**:375, 1904.

49. Hayami, T.: Ueber Aleuronathepatitis, Beitr. z. path. Anat. u. z. allg. Path. **39**:281, 1906.

in the peripheral zone, where the cells were pale and binuclear and contained abnormally bright nuclei. The normal trabeculae were converted into masses of hepatic cells four or more deep. Mitosis was rare, and there was ample evidence of direct division of cells. The lobules became enlarged and irregular in outline. He admitted that occasionally along the course of bile ducts there might be some local proliferation but denied budding or sprouting at their tips. From serial sections, he concluded that these were persistent remains of the finer bile canaliculi. The liver was capable of extreme regeneration provided sufficient reduction in parenchyma had occurred. Regeneration was chiefly compensatory and was the result of proliferation of hepatic cells and not of any transition in cell type.

Whipple and Sperry [32] produced varying degrees of central necrosis by prolonged chloroform anesthesia in dogs. The necrosis was central even after an Eck fistula or a ligation of branches of the hepatic artery had been performed. The liver showed complete regeneration within two or three weeks without scarring or cirrhosis. Necrotic débris was carried off by wandering cells, while the hepatic cells multiplied so rapidly by mitosis that regeneration was usually completed by the eleventh day and always within three weeks. Bile ducts did not take part in the regenerative process. If, however, a wedge was removed from the surface of the liver when the chloroform was administered, the ducts in the adjacent areas would send forth buds and sprouts.

Schultz, Hall and Baker [16] confirmed these results. They produced peripheral necrosis by injecting chloroform into the portal veins of dogs. They found first rapid mobilization of macrophages, which removed the débris, shortly followed by early invasion of fibroblasts and vascular buds. Infection stimulated the formation of fibrous tissue leading to definite cirrhosis. Without infection, repair took place without the interference of connective tissue. Regeneration resulted from proliferation of hepatic cells and also from the newly formed epithelium of the bile ducts. Hepatic cells divided by mitosis. Presumptive evidence of direct division of cells was lacking, namely nuclear fission, and hence, although it was hard to find mitotic figures, mitosis was regarded as the usual method of cell division. The authors attached significance to the transitional type of cell connecting the proliferating bile ducts with newly formed hepatic cells. Mitotic figures were found at the tips of these sprouts. Although the transitional cell might have been a cell either of the liver or of the bile duct, they interpreted it as regenerating from the epithelium of the bile duct since such cells are a less highly differentiated type than the hepatic cell. The uninjured hepatic cells were hypertrophied and multinuclear and were laden with glycogen. Carbon tetrachloride injected into the portal vein was tried with the

production of marked central necrosis. The cirrhosis following the oral administration of carbon tetrachloride was described by Lamson and his co-workers.[50]

Opie and Alford [51] showed that in dogs a diet rich in fat increased the susceptibility of the liver to necrosis following the administration of chloroform, and that a diet rich in carbohydrate had a distinctly protective action. They also noted the increased toxicity of phosphorus in animals on a diet of meat as contrasted with animals on a diet of either carbohydrate or fat.

Davis and Whipple [52] produced central necrosis in the livers of dogs by giving chloroform either as an anesthetic or subcutaneously, and found that regeneration occurred most rapidly on a diet rich in carbohydrate or an ordinary diet of mixed foods. Regeneration was as rapid on a diet of fat as during fasting. Later it was shown that a diet wholly of meat was the equal of a diet rich in carbohydrate in hastening regeneration. After a diet rich in carbohydrate or in protein, in the order of respective efficiency for regeneration came a standard diet, then a diet rich in fat and finally fasting. Moise and Smith [53] verified this work and investigated the toxicity of chloroform and the degree of necrosis with varied diets. They found chloroform most toxic to an animal on a diet rich in fat, and relatively less so to an animal on a standard diet, a diet rich in carbohydrate or a diet rich in protein, in the order named. Repair consisted in the mobilization of leukocytes and the clearing away of débris, accompanied by active regeneration of the cells of the liver, which divided by mitosis.

Bell [54] described regeneration in the livers of dogs when atrophy had first been produced by ligation of the common bile duct, and subsequently had been counteracted by relief of the obstruction by cholecystogas-

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50. Lamson, P. D.; Gardiner, G. H.; Gustafson, R. K.; Maire, E. D.; McLean, A. J., and Wells, H. S.: Pharmacology and Toxicology of Carbon Tetrachloride, J. Pharmacol. & Exper. Therap. **22**:215, 1923; Lamson, P. D., and Wing, Raymond: Early Cirrhosis of the Liver, Produced in Dogs by Carbon Tetrachloride, J. Pharmacol. & Exper. Therap. **29**:191, 1926.

51. Opie, E. L., and Alford, L. B.: The Influence of Diet on Hepatic Necrosis and the Toxicity of Chloroform, J. A. M. A. **62**:895 (March 21) 1914; Influence of Diet on the Toxicity of Substances Which Produce Lesions of the Liver or the Kidney, J. A. M. A. **63**:136 (July 11) 1914.

52. Davis, N. C., and Whipple, G. H.: Liver Regeneration Following Chloroform Injury as Influenced by Various Diets, Arch. Int. Med. **23**:711 (June) 1919; Liver Regeneration Following Chloroform Injury as Influenced by the Feeding of Casein or Gelatin, Arch. Int. Med. **27**:679 (June) 1921.

53. Moise, T. S., and Smith, A. H.: Diet and Tissue Growth. The Regeneration of Liver Tissue on Various Adequate Diets, J. Exper. Med. **40**:13, 1924.

54. Bell, L. P.: The Preoperative Preparation and Surgical Treatment of Carcinoma of the Pancreas with Common Duct Obstruction, California & West. Med. **25**:503, 1926.

trostomy. He found the degree of destruction of parenchymal cells and the extent of proliferation of connective tissue roughly proportional to the duration of obstruction of the common duct. The interlobular capillaries of the bile ducts were stimulated to growth by the dammed-up bile and the increased intrabiliary pressure. With the relief of obstruction, new hepatic cells arose from the undifferentiated bile capillaries and pushed out radially from the peribiliary spaces to form trabeculae with the uninjured hepatic cells. Within two months, the parenchyma of the liver was again normal except for a slight excess of capillaries in the bile ducts.

METHODS OF EXPERIMENTATION

Dogs were chosen for this work because they tolerated the operation well and because an anatomic conformation of the lobes of the dog's liver made removal relatively simple. The animals were kept fasting for twenty-four hours before operation, but water was not withheld. The abdomen was shaved; the skin was cleansed with benzine and with two coats of 2 per cent iodine in ether. All operating was done under general anesthesia.

Operative Procedure.—The operator stood on the right. Through a median line incision from the tip of the xiphoid to just below the umbilicus, a self-retaining retractor was inserted. The left lateral lobe was delivered, and the left coronary ligament severed as the assistant retracted the costal margin. The assistant grasped the pylorus and duodenum in a moist towel with the left hand and exerted gentle even traction downward and to the left. The other lobes were separated from the fragile folds of the peritoneum.

The lobes to be removed were lifted in the left hand and clamped across the pedicle as close to the portal vein as possible. A second clamp (Fenger 1.7-inch [17.78 cm.] curved hemostat) was applied just above the first clamp, being inserted from the opposite side. The cystic duct was held in a mosquito clamp to prevent spilling bile. The abdominal cavity was packed off with gauze. The lobes were grasped firmly to prevent oozing and were severed close to the upper clamp with a scalpel. The pedicle was doubly ligated with linen tape and linen thread doubled. The lower clamp was released as the first ligature was applied. The upper clamp remained in place until the abdomen was sponged free of clot, to guard against slipping. If the small papillary projection of the caudate lobe, lying behind the left central lobe, showed signs of impaired circulation (discoloration or tenseness), it was removed. The peritoneum was dissected from its surface and two curved clamps were applied close to the portal vein. The lobe was severed above the clamps and ligated as before. All fragments of liver were carefully wiped from the pedicles. The abdomen was closed in the routine manner: the peritoneum with linen, the deep and superficial fascia with number 2 iodized catgut, and the skin with linen. The incision was covered with a single thickness of gauze and painted with collodion.

Extirpation of two thirds of the liver gave the greatest impetus to regeneration. McMaster and Rous[55] showed that the left lobes (left lateral and left central) and the gallbladder lobe (right central) together represented from 65 to

55. McMaster, P. D., and Rous, Peyton: The Biliary Obstruction Required to Produce Jaundice, J. Exper. Med. **33**:731, 1921.

70 per cent of the average dog's liver. These lobes and the papillary projection of the caudate lobe were the ones usually selected for removal.

The average weight of the liver was from 3 to 4 per cent of the body weight with an average of 3.4 per cent. In a series of 500 necropsies, Hardenbergh [56] found the weight of the liver to be 4 per cent of the body weight. The variation depends on the age, sex, size of the dog, the length of hair, the breed, general health and diet. By exposing the intact liver below the costal margin at operation, a more accurate estimate of the normal weight could be made. The lobes to be removed were contrasted carefully with the whole liver, the percentage relationship was noted, and then the lobes removed were weighed. Such estimates fell within from 3 to 4 per cent of body weight. These weights were checked against the actual weights of the livers in the cases of death from hemorrhage after injury to the portal vein. A constant relationship does not exist among the various lobes of the liver. In general, the left lateral lobe was the largest and the right central next in size. The other lobes varied even more in their relation to each other.

Mason and Davidson [57] showed that death from autolytic peritonitis ensued after small pieces of liver had been left free in the abdomen. Care must be taken to leave the abdomen clean of all pieces of liver. When the papillary projection of the caudate lobe was left with impeded circulation, the dogs died in like manner. Necropsy showed this lobe to be pale yellow, friable and conspicuously marked. Occasionally, there was local necrosis and hepatitis.

An increased tendency to bleed was not observed after partial hepatectomy. The blood clotted as rapidly after excision of part of the liver as before. Transient jaundice sometimes appeared shortly after operation, lasting from two to five days and then disappearing spontaneously. Congestion sufficient to collapse the capillaries of the bile ducts temporarily may have produced this. And also the congestion may have affected the hepatic cells, thus hindering the removal of bile pigment from the blood.

The dogs were explored at varying intervals and specimens removed from various parts of the remaining lobes. The sections removed were small wedges. Hemorrhage was controlled with figure-of-eight through-and-through sutures of fine catgut, which brought the edges together.

Further tissue was removed, after regeneration had become marked, to secure specimens from the centers of lobes and also to subject the remaining portion to the stimulus for further growth. The technic employed in removing more tissue was the same as that used in removing single lobes from the normal animal.

The animals were killed under ether anesthesia by bleeding from the femoral vessels. The livers were weighed and sections taken from both the surface and the interior of the lobes.

All sections were fixed in Zenker's fluid and a diluted solution of formaldehyde, U.S.P. (1:10). Zenker's fluid gave better results. The sections were stained as a routine with iron hematoxylin and eosin. The sections stained for glycogen were fixed in absolute alcohol and stained with Best's carmine. Those which were stained for fat were fixed in the solution of formaldehyde and stained with scarlet red (Michaelis').

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56. Hardenbergh: Personal communication.

57. Mason, E. C., and Davidson, E. C.: Study of Tissue Autolysis in Vivo; Blood Changes Physical and Chemical, J. Lab. & Clin. Med. **10**:622, 1925.

RESULTS

The restoration of the liver was completed in from six to eight weeks after partial hepatectomy and consisted in hypertrophy of the remaining lobes. This hypertrophy was downward and to the right, filling the right hypogastrium and protruding below the costal margin. The stomach usurped the position of the ablated lobes, displacing the remnant to the right. At first, the surface was glistening, moist and tense, indicative of the congestion within. The edges were rounded, blunt and devoid of notches. The surfaces became flattened, and were yellowish brown instead of the normal reddish brown.

The remaining lobes participated in the hypertrophy in proportion to their relative sizes. By the end of two weeks, the remaining portion of

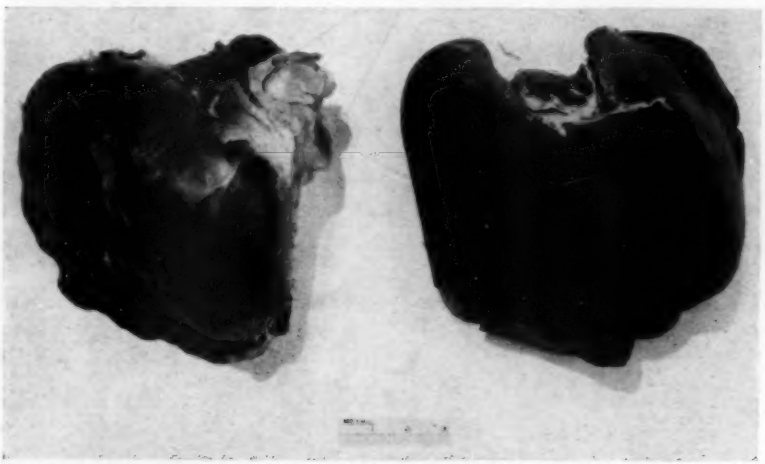

Fig. 1.—A regenerating liver (left), consisting of one lobe, contrasted with a normal liver (right), both dogs weighing the same (10 Kg.).

the liver approximated, in both weight and volume, four fifths of the original organ. The hypertrophy was more rapid when large amounts of tissue were removed. The weight and volume, however, were much the same at the end of eight weeks whether much or little had been extirpated (figs. 1, 2 and 3).

After the liver had been pared down to one lobe by repeated hepatectomy, technical difficulties, concerned with hemostasis, alone prevented indefinite continuation of the process. This regenerative capacity seemed infinite, since the liver tended to regain its normal weight and volume after each partial hepatectomy (figs. 4, 5 and 6).

The liver was extremely congested for from three to five days after operation. The sinusoids were engorged with an excessive blood flow, the equivalent of the supply to the intact liver. This congestion tended

to collapse the terminal bile ducts and may account for the transient jaundice, which disappeared with subsidence of the congestion.

The hepatic cells were swollen, and their pallid cytoplasm was laden with vacuoles. The nuclei were hypertrophic and filled with increased amounts of chromatin. Mitosis in the prophase was noted on the second

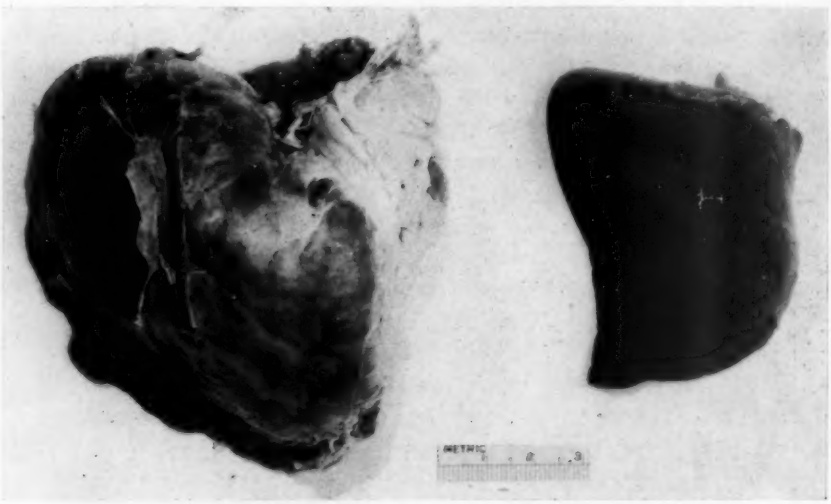

Fig. 2.—A regenerating liver (left) contrasted with the similar lobe (right lateral) of a normal liver (right).

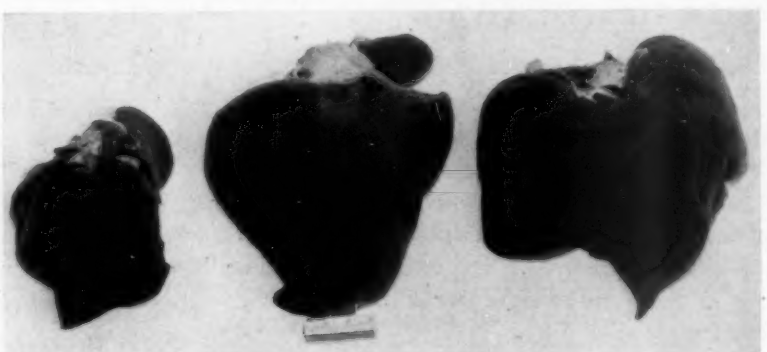

Fig. 3.—A regenerating liver (center) consisting of right lateral and caudate lobes contrasted with a normal liver (right), and normal right lateral and caudate lobes (left), all from dogs weighing 10 Kg.

day. The nuclear membrane and nucleolus were fading, to be replaced by chromatin material arranged in either a thread or a series of fine dots, depicting the spireme in cross-section. By the third day, mitotic figures were seen throughout the lobules, but most frequently in the peripheral

Fig. 4.—The rapidity and extent of the regeneration in from one to fourteen days is shown.

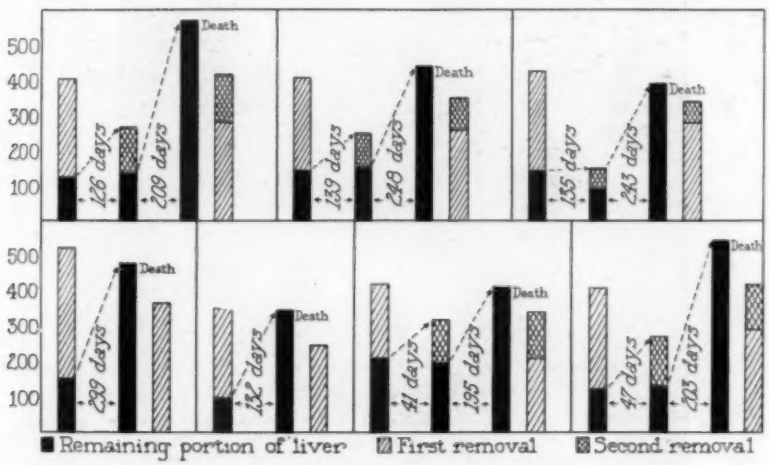

Fig. 5.—The effect of two partial hepatectomies. The impetus to regenerate after the second partial hepatectomy is as great as after the first. The total amount of liver removed in several instances equals the original weight of the organ. The weight of the regenerated liver at death frequently reached the original weight.

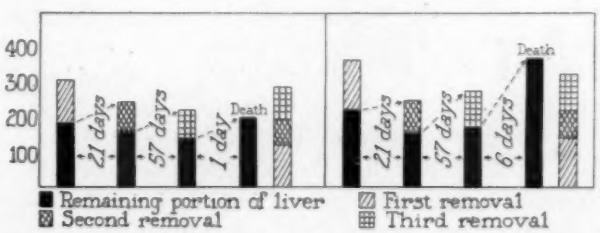

Fig. 6.—The effect of three partial hepatectomies. The tendency to regenerate was present after each partial hepatectomy.

zones, where growth was the most prolific (fig. 7). Although the figures were never numerous, they were the most profuse from the fourth to the tenth day, and were seen as late as the fourth week.

Many binuclear cells were seen at this stage of the process. Cells undergoing nuclear fission were not observed. The nuclei divided rapidly, accompanied by the cells, some early and some later, in definite crops, which accounted for the abundance of binuclear cells shortly after regeneration began. Regeneration was so hasty that the dogs were explored or killed within the first two weeks in order that the restorative process might be observed at its peak. Although the liver reached its optimal weight and volume in two weeks, the histologic picture did not become normal until from six to eight weeks had passed.

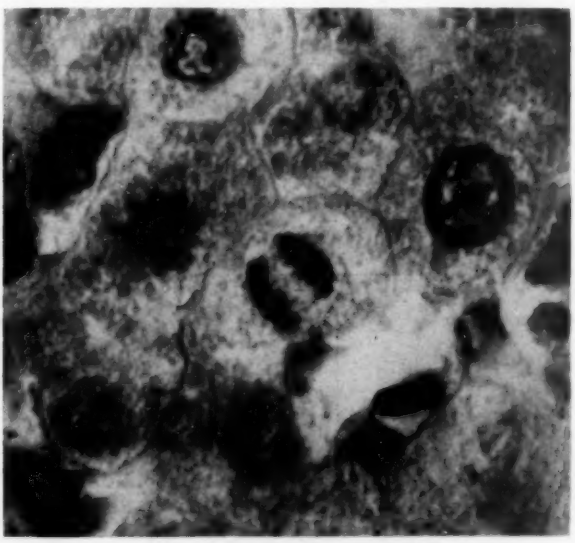

Fig. 7.—A section of liver showing hepatic cells in mitosis, six days after the partial removal of the liver; × 1,450.

Marked proliferation of cells occurred on the fifth and sixth days from the biliary channels in the interlobular areas. These budding areas increased rapidly in size and soon an entire section of the regenerating liver was literally studded with these cytogenic zones, which persisted as late as the sixth week (fig. 8). At the ends of these budding bile ducts appeared new hepatic cells, characterized by large and often double nuclei and by voluminous and more brightly staining cytoplasm. The transition from the interlobular bile ducts, with their basophilic, flattened cells, out through the proliferative buds into regions of newly formed hepatic cells was extremely gradual. The hepatic cells adjacent to these buds stained more basically than normally. Just beyond these transitional cells, however, and continuous with them, were typical hepatic cells with pale pink cytoplasm.

The cells of the proliferating duct buds lacked the deep, basic staining quality associated with the epithelium of normal ducts. From the typical flattened cell of the bile ducts, with its large, dark, oval nucleus, all gradations were seen to the pale, cuboidal hepatic cell with its round nucleus. The transition from one type of cell to the other was so gradual that demarcation was not seen. Mitotic figures were not found in the buds from the ducts, although other observers reported having seen them.

On the basis of their staining reaction, their size and their nucleocytoplasmic ratio, one was impressed with their marked similarity to the adjacent hepatic cells. So pronounced was this resemblance that one must conclude that here were centers for the organization of new

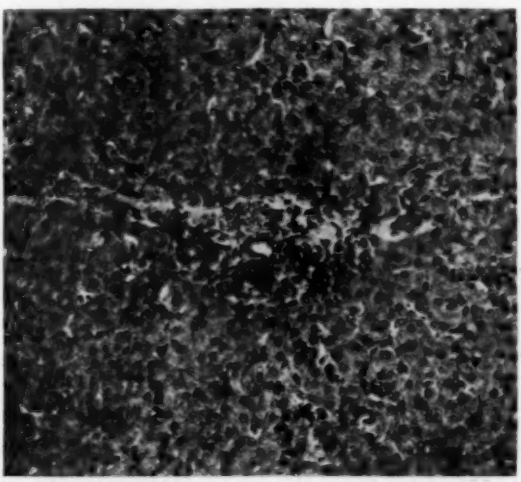

Fig. 8.—Proliferating bile ducts in the midst of areas of new hepatic cells six days after partial hepatectomy; × 150.

hepatic lobules with their trabeculae and related canaliculi. Conclusive proof was, of course, not available within the limitations imposed on any study of fixed sections, and yet the definite budding of the bile ducts, the close similarity of the proliferated cells to those not only of biliary epithelium but of hepatic parenchyma as well, seemed to warrant the conclusion that the new hepatic lobules arose, in part, at least, from the interlobular bile ducts.

The picture was analogous to that seen in the embryonic liver in which the fetal bile ducts branch dichotomously, sending forth buds to form the parenchymal cells.[58] The multitudinous transitional cells so

58. Bloom, William: The Embryogenesis of Human Bile Capillaries and Ducts, Am. J. Anat. **36**:451, 1926.

closely associated with the proliferating bile ducts seemed to indicate another source from which regenerating hepatic cells may arise. The proliferating ducts appeared as clubs and shafts of deeply staining cells, with the lumina visible only here and there. Frequent examples, however, were found of ducts that were sectioned transversely.

The transitional zones and the proliferating buds faded from the third week on. Newly formed bile ducts became well defined, and hepatic cells assumed their typical appearance.

Lobules did not exceed their customary diameter (1 mm.) during regeneration. Although difficult to measure on account of their irregularity of outline, a large series of photomicrographs (after from one day to 189 days) did not reveal any hypertrophy of the lobules. The old lobules gave rise to new lobules at their periphery by budding, as in the fetal liver. The transitional cells, proliferating bile ducts and mitotic figures were found chiefly in the peripheral zones, where growth was most active.

The participation of the bile ducts in regeneration maintained the normal ground plan of the liver. If only the hepatic cells multiplied, the lobules would have to hypertrophy to accommodate them, but this did not seem to be the case. Bile ducts proliferated and appeared to send forth shoots of transitional cells which blended with newly formed hepatic cells to preserve the normal architecture. In spite of the irregularity of lobular outline, there was sufficient similarity in contour to enable one to recognize clearly that they maintained uniformity in size during and after regeneration.

Mitosis of the hepatic cells, seen on the second day, was the first evidence of regeneration. Proliferation of the bile duct appeared on the sixth day and persisted until the sixth week. After two months, a section of regenerated liver could not be distinguished from normal liver. Rarely an interlobular bile duct was seen which had preserved its proliferated lining. The architecture was that of normal hepatic tissue and the cells were identical in every respect with normal hepatic cells.

The sinusoidal endothelium kept pace with the regenerating hepatic cells. The endothelium accompanied the newly formed hepatic cells as they attained trabeculation. Since the remnant of liver had to tolerate the blood supply of the normal liver, the growth of the endothelium might be viewed in the light of Thoma's hypothesis that an increase of blood pressure in capillary areas leads to the formation of new capillaries.

Cells of the liver, particularly those in the hyperplastic peripheral zones, were laden with glycogen from the first to the fourth week after partial removal of the liver. The glycogen occurred as large vacuoles in the clear cytoplasm of regenerating cells. Some cells appeared to

be completely filled with glycogen, their nuclei lacking or undergoing a degeneration suggestive of malnutrition subsequent to the early congestion.

Immediately after operation, fat was found in large quantities, in droplets of varying sizes throughout the lobules, and was especially abundant in the marginal zones of the lobules and the periportal connective tissue. Between the first and fourth days, the hepatic cells contained fat in small droplets. The increase occurred rapidly on the first day and continued for three days; then the fat content fell and the normal level was regained two weeks after operation.

COMMENT

The term "regeneration" was retained because replacement of hepatic tissue has been known by that name since reports of it first appeared in medical literature. The process does not fulfil the biologic requirements of regeneration in that replacement of the tissue removed does not take place at the site of removal. There is, however, definite restoration of lobules, the functional units of the liver, and of hepatic cells. The functioning of the liver is normal throughout the process [41] and there is always bile in the duodenum.

Morgan [59] classified regeneration as of two types, the first, "physiologic regeneration," which is the wear-and-tear sort encountered in the life cycle of an organism; and second, "restorative regeneration," which comprises the restoration of organs after their pathologic alteration. He excluded this process from either category, regarding it as one of pure hypertrophy, on the basis of Ponfick's conclusion that the lobules hypertrophy. It was suggested that this process might properly be called "compensatory hyperplasia."

The chief factor in restoration of the liver is the intense and rapid multiplication of hepatic cells. The participation of the budding bile ducts, which give rise to transitional cells and they, in turn, to new hepatic cells is, however, of definite assistance in regeneration and is chiefly responsible for the preservation of the normal histologic ground plan.

The lobes left behind at operation are the site of regeneration. They subsequently undergo marked hypertrophy since they contain the newly formed hepatic cells and lobules. The volume of these lobes, when regeneration is complete, approximates that of the original liver. The stump where a portion of the liver was removed does not show evidence of regeneration.

Cells of the liver divide by mitosis. The increased number of binuclear cells does not, alone, warrant the assumption that a direct division

59. Morgan, T. H.: Regeneration, New York, The Macmillan Company, 1901.

of cells has occurred. Certain observers [60] believe this to be so because of the scarcity of mitotic figures. Conclusive evidence of amitosis (cells undergoing nuclear fission) is lacking. Although scant in numbers, sufficient mitotic figures are seen to justify the conclusion that this is the characteristic manner of division.

Ponfick, von Meister and Schultz, Hall and Baker noted the early appearance of numerous binuclear cells, filled with delicate, pale protoplasm typical of the newly formed cells. Ponfick suggested that the cells divide promptly in a definite series of crops, some early and some late, until finally the number of binuclear cells has been reduced to normal (about 5 per cent). Schultz, Hall and Baker described this process as one of "cytoplasmic lag."

Mitotic figures have not been found in any bile ducts. Ponfick,[31] Meder,[8] von Meister,[7] MacCallum [10] (1902) and Schultz, Hall and Baker,[16] however, noted them in proliferating bile ducts.

An interesting question concerns the extent of participation of bile ducts in the regeneration of hepatic cells. Marchand,[17] Ribbert [18] and Muir [12] denied the proliferation of bile ducts in human beings, and attributed the apparent proliferation to either degeneration or regeneration and rearrangement of the hepatic cells in an effort to preserve their continuity. On the other hand, Meder,[8] Carraro,[19] Stroebe,[20] Barbacci,[9] MacCallum,[10] Hess,[14] Hess,[23] Miller and Rutherford [22] and Herxheimer and Garlach [15] all believed that bile ducts proliferate and, excepting Carraro,[19] believed that these proliferating ducts served as a source for the growth of new hepatic cells, at least under conditions of stress. They found the first evidence of regenerative activity in the proliferating bile ducts. Most textbooks agree on this point.

As regards the proliferation of bile ducts induced experimentally, Milne [13] alone denied that it was constant and asserted that its occurrence was adventitious. Von Podwyssozki,[6] Ponfick,[31] von Meister,[7] Flöck,[42] Porcile,[48] Hayami,[49] Schultz, Hall and Baker [16] and Bell [54] believed that bile ducts proliferate and all except Ponfick, von Meister and Flöck gave to the budding bile ducts a definite part in the regeneration of hepatic cells. Whipple and Sperry [32] found proliferation of bile ducts only when they cut wedges from the surface of the liver and not from prolonged chloroform anesthesia by itself. Herxheimer and Garlach,[15] with serial sections, showed all the gradations from the typical bile duct, through the zone of proliferation and transitional cells, to the newly formed hepatic cells.

The consensus of opinion is that bile ducts proliferate and serve as a source of new hepatic cells, especially when the destruction of parenchyma has been severe. Since 70 per cent of the liver was removed

60. Milne (footnote 13). Miller and Rutherford (footnote 22).

in these experiments, the remaining portion might revert to any available source of aid in regeneration. This conclusion is warranted by the appearance of large numbers of buds of the bile ducts on the sixth day, which persisted until the seventh or eighth week.

The lobules of the liver do not hypertrophy during regeneration. Comparatively low-power photomicrographs of sections removed at varying intervals did not reveal variation from normal. Although Ponfick,[31] von Meister [7] and Flöck [42] believed that lobules hypertrophied, Mall [43] showed clearly that the process was not one of hypertrophy but rather of the formation of new complex lobules, and that the so-called clover-shape and heart-shape lobules represented an old lobule surrounded by newly formed lobular buds. This budding was extremely rapid, and the profusion of new hepatic cells, combined with the irregularity of the lobules, makes the picture confusing.

Mitotic figures are more numerous in the peripheral zones than elsewhere in the lobules. Ponfick [31] and von Meister [7] noted this and regarded it as further indication of lobular hypertrophy. The peripheral zone is the region of proliferating bile ducts, transitional cells and recently divided hepatic cells, the site where new lobules are forming. It is impossible to distinguish new lobules from old lobules, since both are penetrated by proliferating bile ducts. In short, the regenerating liver presents a picture of uniform, although irregular, lobules with growth most active at their peripheries in the region of the budding bile ducts.

The ability of the liver to regenerate is potentially infinite. Von Meister [7] first made this observation. The liver continues to restore itself after partial hepatectomy for the second or even the third time exactly as it does after the first. Technical difficulties concerning hemostasis impose limitations on carrying this process forward indefinitely. When the liver has been pared down to one greatly hypertrophied lobe, further resection is impossible since the capsule of Glisson is much too friable to hold any sort of hemostatic suture.

SUMMARY

When from one fifth to three quarters of the liver has been removed from a dog, the remaining part regenerates completely in from six to eight weeks. The process consists of marked hypertrophy of the remaining lobes. The pedicle does not regenerate. Restoration takes place within remaining lobes which hypertrophy until they attain at least four fifths of the size and weight of the original liver.

Extreme congestion of the sinusoids is the earliest change in the liver. The cells become swollen and pale. Mitotic figures appear in the peripheral zones of the lobules as early as the second day after partial hepatectomy, but are most numerous from the third to the sixth day.

Many binuclear cells are present shortly after operation, but evidence of amitotic division, namely nuclear fission, is lacking. The so-called cytoplasmic lag is offered in explanation of this seeming contradiction. The nuclei divide promptly by mitosis, but the cytoplasm permits the lapse of varying periods of time before it separates into the daughter cells.

Regeneration closely resembles the embryonic development of the liver. The chief cellular activity is at the periphery of the lobules, where bile ducts send forth buds of proliferating cells. Although hepatic cells are the main source of new tissue, buds of the bile ducts seem to play a definite part in regeneration of the liver. The transition from these proliferating duct cells to new hepatic cells is very gradual. The stress of regeneration has caused reversion to the primitive mode of production of hepatic cells in which the undifferentiated capillaries of the bile ducts give rise to new hepatic cells by dichotomous branching.

The other elements of the liver, including the connective tissue, the sinusoidal endothelium and the lymphatic endothelium, are carried along on the wave of growth without appreciable delay.

There is a marked increase in the glycogen content of the liver, especially in the periportal zones, from the first to the fourth weeks after partial hepatectomy. Glycogen is contained in large vacuoles in the cytoplasm. The fat content of the liver increases for four days and then falls slowly until the normal content is again reached ten days later.

At the end of from six to eight weeks, sections reveal tissue indistinguishable from normal liver, with the possible exception that proliferating bile ducts rarely persist.

The lobules do not hypertrophy but undergo a hyperplastic, budding process at their periphery, productive of new lobules so similar in size and shape that they cannot be distinguished from the old ones. Here, again, the analogy to the embryologic development of the liver is evident.

The liver appears to possess an infinite capacity for regeneration. The remaining part of the liver responds as rapidly and completely after the second or third partial hepatectomy as after the first.

ILLUSTRATIVE PROTOCOL

A white and brown bull terrier, weighing 11.7 Kg., was operated on Jan. 27, 1926, under ether anesthesia. The left lateral, left central and right central lobes of the liver were removed, weighing respectively 122, 56 and 92 Gm., or a total of 270 Gm., which was approximately 70 per cent of the original amount of tissue, estimated on this basis to be 386 Gm. Uneventful recovery followed.

Thirty-five days later, the animal (then weighing 11.6 Kg.) was explored. A specimen of liver was removed from the caudate lobe, and was fixed in Zenker's fluid and a diluted solution of formaldehyde U.S.P. (1:10). The right lateral and caudate lobes, which remained, were markedly enlarged, their edges were rounded, and the lower border of the liver was well down below the costal margin on the right. The animal was in good condition.

On June 2, 1926, 126 days after the first operation, the animal, then weighing 13.5 Kg., was explored. The right lateral lobe, weighing 132 Gm., was removed. This was equivalent to 50 per cent of the total amount of liver at this time.

The animal was explored again 176 days after the first operation. The dog's weight was 12.8 Kg. The caudate lobe, now the only remaining one, showed marked hypertrophy. A wedge-shaped section was removed from the edge of the lobe.

On Feb. 16, 1927, 385 days after the first partial hepatectomy, the animal (then weighing 11.9 Kg.) was killed under ether by bleeding. The caudate lobe appeared as two lobes due to the marked enlargement of the papillary projection of the caudate lobe. The liver weighed 570 Gm., and was macroscopically normal in color and consistence. Histologically, the section showed normal hepatic tissue. Lobular hypertrophy or bile duct proliferation could not be seen.

MICROSCOPIC CHANGES OF MUSCLE IN MYOSTATIC CONTRACTURE CAUSED BY TETANUS TOXIN *

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When an appropriate dose of tetanus toxin is injected into one limb of a highly resistant animal, such as the rabbit or the rat, only the muscles of the leg which receives the injection are affected. When this local tetanus is fully developed, the limb is held continuously in rigid extension. In the early stages, the limb relaxes when the animal is anesthetized; but in from five to seven days after the injection, if the dose has been an adequate one, the muscles become set in this posture of rigid extension and fail to relax under deep anesthesia or even after section of the motor nerve (Meyer and Ransom,[1] Ranson and Morris [2]). By section of the nerve, the muscles are put at rest, yet they remain shortened and are able to support considerable loads without being drawn out to their original lengths.

To this shortened state of resting muscle, we have applied the term myostatic contracture [3] to differentiate it from the hypertonic contractures, which are maintained by a continuous stream of nerve impulses into the affected muscles. Familiar examples of muscles that have acquired a shorter than normal resting length are furnished by the contractures that restrict the movements of joints after immobilization of the latter for weeks in plaster casts, the permanent shortening of muscles after the division of their tendons and, in their early stages at least, the paretic contractures due to the unequal paralysis of antagonistic groups of muscles in anterior poliomyelitis and multiple neuritis.

It is not easy to understand why a muscle cut off from its innervation should remain shortened. A preliminary histologic study showed that the myostatic contracture of local tetanus was not due to fibrosis (Ranson and Sams [3]). It was thought that there might be an accumulation of lactic acid in the muscle in tetanus, and that the failure to

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1. Meyer, H., and Ransom, F.: Arch. f. exper. Path. u. Pharmakol. **49**:369, 1903.

2. Ranson, S. W., and Morris, A. W.: J. Comp. Neurol. **42**:99, 1926.

3. Ranson, S. W., and Sams, C. F.: J. Neurol. & Psychopath. **8**:304, 1928.

relax might in this respect resemble the delayed relaxation of fatigued muscle. But an investigation of this question showed that the lactic acid content of muscles in the contracture of tetanus was not above normal.[4]

In the hope of gaining some further insight into the nature of the contracture, we undertook a detailed study of the histologic changes in the tetanic muscles. So far as we were able to learn, this had not been attempted before. Most investigators who had studied the pathology of tetanus had directed their attention to the changes in the nervous system; and those who had examined the muscles had been concerned either with local inflammatory reactions produced by the tetanus bacillus (Stangl[5]) or with muscles obtained at autopsies in fatal cases of general tetanus (Wisbaum[6]). Since, in general tetanus, myostatic contracture seldom develops, such material could contribute nothing to the solution of our problem.

Wisbaum studied sections of the psoas and the quadriceps obtained at autopsy in a case of general tetanus complicated by terminal pneumonia. Hemorrhagic areas were found among the muscle fibers. Within these areas were the remains of degenerating muscle fibers. Large mononuclear cells, rich in cytoplasm, with lightly staining nuclei, were scattered through the hemorrhagic areas. Nuclear proliferation was seen in some of the adjacent muscle fibers. These muscle nuclei bore a close resemblance to those of the mononuclear cells in the hemorrhagic areas. In cross-sections typical pictures of waxy degeneration could be seen. These changes were similar to those seen by Forbus[7] in the rectus abdominis after death from pneumonia. Hence, it is not clear what part if any of the alterations described by Wisbaum were due to the action of tetanus toxin.

METHOD

In our work, local tetanus was produced in rats by the injection of tetanus toxin into the muscles above the popliteal space of the right hind leg. The dose of toxin necessary to produce local tetanus had to be determined by experience with the particular strength of toxin used, since it is subject to some deterioration. When freshly prepared, the toxin, which was kindly furnished us by the Research Laboratory of Parke, Davis & Company, was standardized so that 0.001 cc. constituted a minimum lethal dose for a guinea-pig. Even when fresh, 0.005 cc. of this toxin was required to produce high grade local tetanus in rats weighing 150 Gm. As the toxin aged, this had to be increased up to 0.04 cc. Two days

4. Davenport, H. A.; Davenport, H. K., and Ranson, S. W.: J. Biol. Chem. **79**:499, 1928.

5. Stangl, F. H.: J. Infect. Dis. **31**:22, 1922.

6. Wisbaum, K.: Deutsche Ztschr. f. Nervenh. **80**:75, 1923.

7. Forbus, W. D.: Pathologic Changes in Voluntary Muscle: Degeneration and Regeneration of Rectus Abdominis in Pneumonia, Arch. Path. **2**:318 (Oct.) 1926.

after the injection of the toxin, the patellar tendon was cut to permit flexion of the knee and complete contraction of the gastrocnemius.

The earliest stages of local tetanus in which myostatic contracture had developed were studied, the criterion for determining its presence being the failure of the muscle to relax under deep ether anesthesia. In six of the rats, contracture developed in five days from the time of injection; in the remainder, it required seven or eight days for the toxin to produce the permanent contracture desired.

In order to eliminate artefacts as much as possible, the method of removing and fixing the muscles was varied. It was found that the best preparations were obtained when the gastrocnemius muscle was dissected out immediately after the animal had been killed with ether, and suspended in a moist chamber under a tension of 25 or 50 Gm. for one hour before being placed in the fixing fluid with the weight still attached. This interval allowed between death and fixation prevented the twitching that usually takes place when a recently removed muscle is placed directly in fixative. Straight fibers were thus obtained. Three fixatives, Bouin's solution, Zenker's fluid and a diluted solution of formaldehyde U. S. P. (1:10) were used in order that several stains might be employed. The stains were hematoxylin and eosin, iron hematoxylin, van Gieson's picrofuchsin with Delafield's hematoxylin, Mallory's triple connective tissue stain and Mallory's phosphotungstic hematoxylin.

Paraffin sections of the right (tetanic) and the left (control) gastrocnemii 8 microns thick were mounted on the same slide to insure uniformity of treatment. Although in rats with tetanus the muscles in which no injections were made appeared little altered, it was thought best to use the gastrocnemii of normal animals for controls, as well.

It was found that Mallory's triple stain gave the best definition of striation, as well as differentiation of connective tissue. Hematoxylin-eosin and van Gieson's stain showed nuclear and degenerative changes most clearly. Iron hematoxylin and phosphotungstic hematoxylin were used more for checks on changes noted with the other stains.

Longitudinal sections and cross-sections were examined for changes in the staining of the tetanic muscles with the different dyes, for nuclear changes, for difference in diameter between fibers of the tetanic muscles and those of the control side of the same animal, for degenerative changes and for changes in the striations. Enlarged photomicrographs of cross-sections of the tetanic and of the control muscles were used for measuring the diameters of the fibers. The fibers of four areas on the tetanic side and four comparable areas on the control side were measured. Two measurements, one through the greatest and one through the smallest diameter, were made of each fiber and an average of these taken as the approximate diameter of the fiber. The striations were measured directly with the use of a filar micrometer and the oil immersion objective. In a given portion of a fiber, consecutive isotropic and anisotropic bands were measured for as great a distance as the stripes remained parallel. Such measurements were made on tetanic and control muscles of the same rats and on muscles of normal rats. Table 2 shows only averages of such consecutive measurements in given areas.

In six of the experiments, the muscles were measured after fixation in a diluted solution of formaldehyde U. S. P. (1:10) under a tension of 25 Gm. The length of the muscle and the length of a definite, easily identifiable bundle of parallel fibers was determined.

RESULTS

Changes in Length of Muscles.—Measurements of the muscles after fixation in formaldehyde showed that the right (tetanic) gastrocnemii were on the average 24.5 mm. in length, which was 4.6 mm. shorter than the average length of the control muscles of the left leg. The average length of the muscle fibers in the particular bundle of parallel fibers that was measured was 4.1 mm. on the side into which injections were made, which was 4.5 mm. shorter than the same group of fibers on the control side. The length of the muscle had decreased 15 per cent and that of the fibers 52 per cent. The apparent discrepancy between the percentage of shortening of the whole muscle and that of individual fibers can be accounted for by the oblique course of the fibers through the muscle, and possibly also by the stretching of the fibrous septums to which the muscle fibers are attached.

TABLE 1.—*Differences in Diameter Between Fibers of Tetanic Muscles and Those of Normal Muscles*

Right Leg (Tetanus)			Left Leg (Control)		
Area	Number of Fibers Measured	Average Diameter in Microns	Area	Number of Fibers Measured	Average Diameter in Microns
I.....	138	31	I.....	157	24
II.....	167	31	II.....	152	25
III.....	183	28	III.....	180	22
IV.....	58	40	IV.....	58	34
Average.....		32	Average.....		26

The weights of the gastrocnemii were not materially affected, the average being 1 Gm. for the side into which injections were made as compared with 0.99 Gm. for the control side.

Changes in Diameter.—Corresponding to the decrease in length of the tetanic muscle fibers there was, as might have been expected, an increase in diameter. This is well illustrated in figure 1, *A* and *B*. The fibers appear larger, more rounded and separated from one another, while in the normal muscle they are closely packed together and have angular outlines. Measurements of the diameters of fibers on the tetanic and the control sides of rat T-XXVII made in the manner described, gave an average diameter of 26 microns for the fibers of the control muscles and 32 microns for those of the tetanic side. Table 1 shows the differences between the two sides in the four comparable areas measured.

Changes in Striation.—In longitudinal sections, the fibers showed a blurring of the cross striations. This will be evident when parts *D* and *E* are compared with *C* in figure 1, and *B* and *C* with *A* in figure 2. In the normal muscle fiber, the myofibrils are bound tightly together and held in exact alinement so that the dark disks (*Q*) separated by the light disks (*J*) in all the fibrils lie in the same planes, thus producing the

EXPLANATION OF FIGURE 1

Fig. 1.—*A* represents a transverse section of the medial head of the left (control) gastrocnemius of rat T-XXVII; × 100. *B* represents a transverse section of the medial head of the right (tetanic) gastrocnemius of rat T-XXVII; × 100. The portions illustrated in *A* and in *B* were taken from corresponding areas of the two muscles. *C*, a longitudinal section of normal muscle (gastrocnemius, rat N-XIX). Mallory's triple stain after Bouin fixation; × 285. *D*, a longitudinal section of tetanic muscle (gastrocnemius, rat T-XXVIII) showing mottled staining in two fibers and blurred striations without irregular staining in the center fiber. Mallory's triple stain after Bouin fixation; × 285. *E*, a longitudinal section of tetanic muscle (gastrocnemius, rat T-XXVIII). Fibers *a* and *c* show mottled staining with striations pulled out of alinement to some extent. Fiber *b* shows blurring of the cross striations without irregularity in staining. In such a fiber the myofibrils have been pulled out of alinement to such a degree that the striations are continuous across only small groups of fibrils. Mallory's triple stain after Bouin fixation; × 285. *F*, a longitudinal section of tetanic muscle (gastrocnemius, rat T-XVII). Fiber *a* shows darkly staining areas with a light stretched area between. In such stretched areas, the isotropic bands are wider and the *J*/*Q* ratio increased. Striations can be seen in parts of the dark areas, but they are not distinct enough to measure. Mallory's triple stain after Bouin fixation; × 400. *G*, a longitudinal section of tetanic muscle (gastrocnemius, rat T-XVII). Fiber *a* is similar to *a* in figure 1 *F*. Fiber *b* shows a darkly stained granular area with light regions, which also appear to be disintegrating, on each side of it. It seems probable that this represents a later stage in the same process of alteration that is taking place in figure 1 *F*, *a* and in fiber *a* of this figure. Mallory's triple stain after Bouin fixation; × 400.

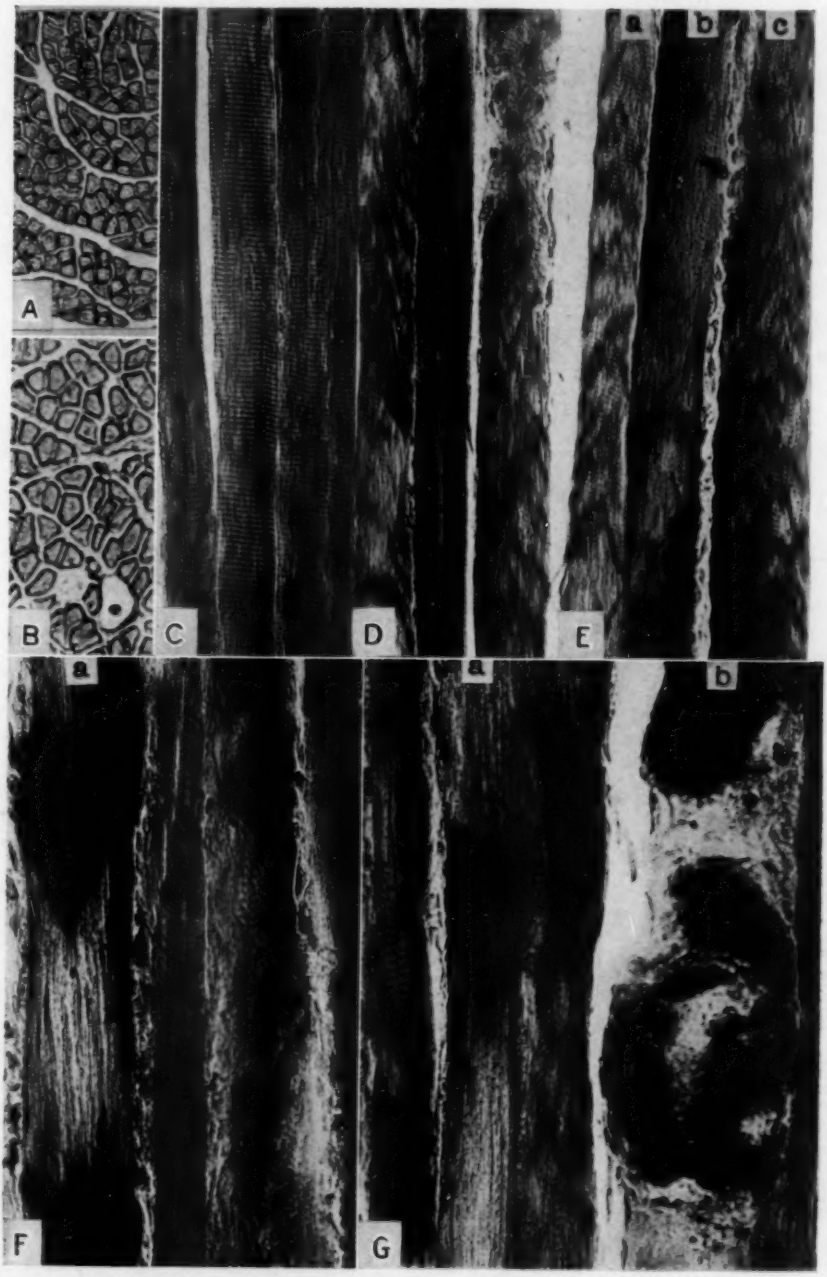

Figure 1

EXPLANATION OF FIGURE 2

Fig. 2.—*A* represents a longitudinal section of normal muscle (gastrocnemius, rat N-XIX) showing the orderly arrangement of muscle nuclei with their long axes parallel to the long axes of the fibers. Hematoxylin and eosin; × 145. *B*, a longitudinal section of tetanic muscle (gastrocnemius, rat T-XXVII) eight days after the injection of toxin, showing an aggregation of nuclei within fiber *a*. Note that the fiber is continuous with the nucleated area at each end. The two fibers above *a* show some increase in the number of muscle nuclei. The nuclei of most of the fibers illustrated here do not have the normal arrangement with reference to the long axes of the fibers. Hematoxylin and eosin; × 145. *C*, a longitudinal section of tetanic muscle (gastrocnemius, rat T-XVII) seven days after the injection of toxin. The animal died of general tetanus at this time. Fiber *a* shows a granular area with an increase in the number of nuclei around the edges of it. *b*, *c* and *d* are fibers with pronounced longitudinal striation. Here also may be seen the marked dark and light staining and an apparent loss rather than increase in the number of nuclei in parts of the fibers. Hematoxylin and eosin; × 145.

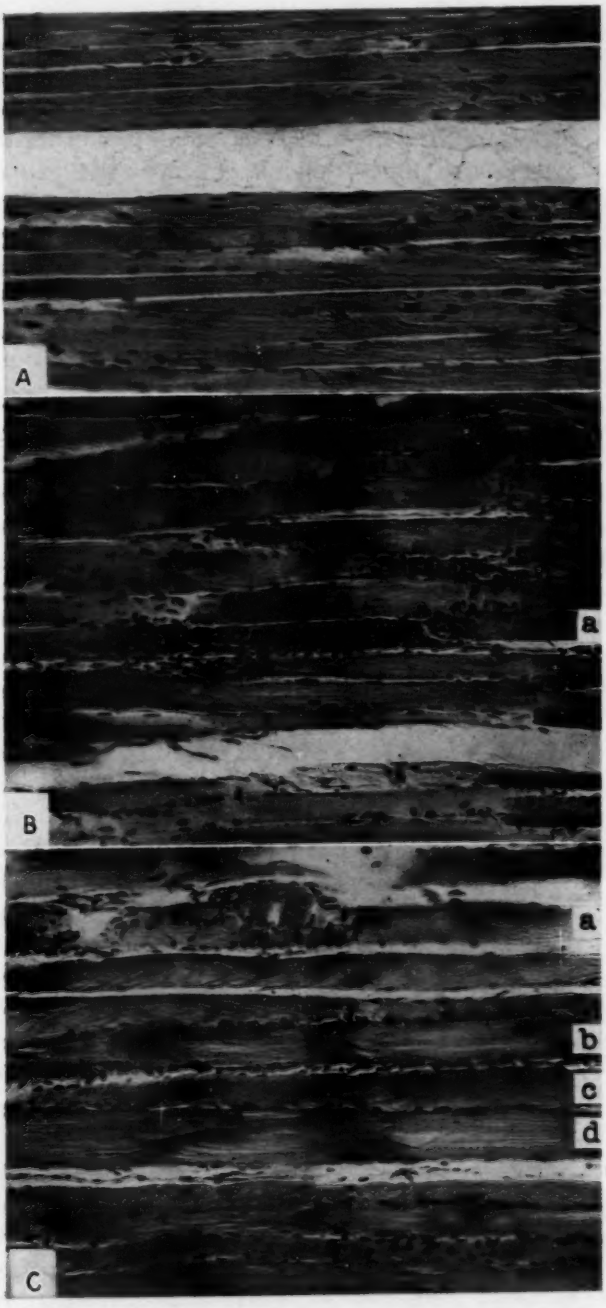

Figure 2

transverse striations. The ground membranes of Krause, designated by the letter *Z*, are supposed to extend across the fiber binding the fibrils together. If this is the case the interfibrillar portions of these membranes must be dissolved or disrupted in some of the fibers in the contracture of tetanus. At any rate, it is certain that in such fibers the individual myofibrils separate from one another and are more easily seen than in normal muscle. Instead of being straight and parallel to the long axis of the fiber, they are bent on themselves, giving the fiber an irregular but rather pronounced longitudinal fibrillation (as may be seen in fiber *d* in fig. 2 *C* and in fiber *a* in fig. 1 *E*). As a result of these changes, the dark disks in adjacent fibrils lose their exact transverse alinement and the striations become obscured (as in *b* in fig. 1 *E*).

Changes in Staining.—The most striking histologic change found in all specimens of tetanic muscle was an irregular mottling of the fibers, in which there appeared dark and light staining areas of irregular contour, varying considerably in width. In some instances, the anisotropic elements seemed to have lost their staining property, producing the irregular light fields, while some areas of the fiber retained it, giving the dark fields (fig. 1, *D* and *E*). In others, the dark areas stained darker than normal, while the light areas seemed to have lost either in part or entirely the staining property of the anisotropic bands (*Q*), the *z* line remaining faintly visible. In some of the cases in which the dark areas were darker than the normal staining of a fiber, the *Q* bands appeared to be crowded close together, almost obscuring the isotropic bands. In others, there was normal spacing, so that the dark staining here could not be accounted for by approximation of anisotropic bands. Parts of some of these extremely dark areas appeared homogeneous, with the striations distinguishable only where they joined the light ones. In fibers so stained, the light areas between dark ones appeared to have been stretched (as in *a* in fig. 1, *F* and *G*).

Irregular staining was shown with all the dyes used. It was a matter of dark and light shading of the same color in sections stained with hematoxylin and eosin, picrofuchsin, Mallory's triple stain after formaldehyde or Bouin fixation and phosphotungstic hematoxylin. In sections stained with iron hematoxylin, the light areas appeared unstained or lightly stained, while the dark ones were dark blue or black.

Mallory's triple stain after Zenker fixation stained the anisotropic bands of the normal muscle blue and the isotropic bands orange, the *z* line appearing brown. In tetanic muscle, the same areas that stained light with other stains were orange and the dark areas were blue. The *Q* bands remained visible as brown stripes in the orange fields after they no longer stained blue. This would indicate that although the anisotropic disk may change in its staining reaction, it

retains its normal relation to Krause's membrane. Nageotte [8] obtained a similar picture from muscles in which contracture had been produced by electrical stimulation. He interpreted this mottled appearance as a contraction of the fiber in spots, for the areas between the dark areas showed striations at the usual normal spacings. He believed that in the parts of the dark fields where striations could not be seen, the dark disks had drawn so close together that the space between them became invisible, since very fine striation was present in the dark areas if the muscle had been pulled out slightly before being fixed. With contracture due to chloroform, he [9] obtained histologic pictures that corresponded to various stages of faradic contraction. In early contraction due to chloroform, the contracted areas were small and finely striated; in later stages, they were larger and more like the extreme faradic contracture.

That the change in staining reaction observed in longitudinal sections was not merely on the surface of the fibers was shown by transverse sections. Here one might see fibers that were spotted with light and dark areas. A transverse section through almost any plane in fiber *a*, figure 1 *E*, would be expected to show spotted staining. On the other hand, fiber *b* in figure 1 *E* should show a cross-section practically uniform in color. A transverse section through the light part of *a* in figure 1 *F* would be lighter than normal, though probably uniform in color, while one through either of the wide dark areas of the same fiber should be uniform also, but darker than normal. In almost any single cross-section of tetanic muscle all of these variations could be seen.

In our preparations, a measurement of isotropic and anisotropic bands in the dark and light areas in different stages of development, indicated that mottling may appear before the relationship between the widths of the isotropic and anisotropic disks and the sarcomere length changes, for in some fibers, such relationships were found to be the same as those found in normal muscle. In later stages, however, the relationships were altered. Measurements were made in six normal muscles, and it was found that the sarcomeres varied in length from 2.5 to 2.9 microns, the *J* disk from 1.26 to 1.5 microns, the *Q* disk from 1.27 to 1.46 microns and the *J*/*Q* ratio from 0.86 to 1.03. The muscles in the contracture of tetanus did not show any considerable variation in the *J*/*Q* ratio except in the stretched light areas, where this ratio was greatly increased, and possibly in the dark adjacent areas, which could not be measured.

As can be seen from figure 1 *D*, *E*, *F* and *G*, the contracture of tetanus did not present a uniform picture. The great distortion of the fibrils in many of the fibers prevented the measurement of the striations.

8. Nageotte, J.: Compt. rend. Acad. d. sc. **180**:761, 1925.

9. Nageotte (footnote 8, p. 1963).

Many of the measurements made in less distorted areas did not show any variation from the normal. Such measurements as it was possible to make in fibers showing different degrees of irregular staining showed variations ranging from normal relationships of isotropic and anisotropic bands in both light and dark staining areas, to a narrowing of the isotropic bands and consequent shortening of the sarcomere in the dark areas, and either a normal sarcomere length and J/Q ratio, or a lengthened sarcomere resulting from an increase in the width of the isotropic bands in the light areas. All these variations have been recorded in table 2, in which an example of the average widths of the Q and J disks in adjacent light and dark staining areas of the same fiber has been taken from each of four different animals. In rat T-XXII, the lengths of the sarcomeres and the J/Q ratios in both

TABLE 2.—*Average Widths of Isotropic and Anisotropic Disks in Comparable Areas of Irregularly Staining Fibers in Tetanic Muscles from Four Different Animals*

Rat*	Type of Area	Sarcomere (Microns)	J Disk (Microns)	Q Disk (Microns)	J/Q Ratio
T-XXII	Dark	2.6	1.15	1.40	0.82
	Light	2.5	1.22	1.30	0.94
T-XXIII	Dark	2.4	1.10	1.35	0.81
	Light	2.3	1.05	1.27	0.83
T-XXVIII	Dark	2.2	0.91	1.31	0.69
	Light	2.6	1.32	1.30	1.01
T-XVII	Stretched (light)	3.7	2.37	1.30	1.82
	Stretched (light)	3.6	2.20	1.28	1.72
	Stretched (light)	3.3	2.00	1.26	1.59
T-XXVIII	Stretched (light)	3.2	1.82	1.34	1.36

* In the first three animals listed, the light and dark staining areas were adjacent and in the same fiber. The dark areas adjacent to stretched light areas could not be measured, so that for these there are no corresponding measurements in dark areas.

the light and the dark areas of the same fiber were within the range of values found in normal muscle. Rat T-XXIII showed somewhat shortened sarcomeres and a slightly lowered J/Q ratio in both the light and the dark areas. Rat T-XXVIII, however, showed a difference in the relationships of the widths of the J and Q disks as between the dark and the light areas as follows: The sarcomeres of the dark field were shortened as a result of the narrowing of the J disks, the Q disks remaining normal in width. The adjacent light area, however, showed normal sarcomere lengths and normal J/Q ratio. The stretched light areas of the muscle in rat T-XVII and rat T-XXVIII showed marked increase in sarcomere length and in the width of the J disk. Here, also, the Q disk retained its normal width, so that it seemed from these figures that the variations occurred in the J disks rather than in the Q disks. In those fibers in which the light areas were stretched and the dark ones were unusually compact, it was not possible to measure the striations in the dark ones. Though striations could be

seen in many of the dark regions, they were greatly distorted, or the definition was poor owing to the deep staining. It could not be determined by measurement, therefore, whether or not a shortening of sarcomeres had taken place in the dark regions which was proportional to the amount that they were shown to have lengthened in the lightly stained areas.

According to one theory (Jordan [10]), contraction in striped muscles is associated with a genuine reversal of striations as regards the deeply staining substance of the *Q* bands. These dark bands split into two halves, which separate and approach the *z* membranes that limit the sarcomeres at either end. In this way, contraction bands are formed, each of which is composed of the fused opposite halves of two adjacent *Q* bands. We have not been able to find any evidence of such contraction bands in our preparations of the tetanic muscle. Certainly, the dark areas that sometimes stretched bandlike across a fiber (as in *a* of fig. 1 *F*) were not contraction bands in this sense, since they were much too wide, and since, under favorable conditions, transverse striations could be seen in them.

Measurements of striations in the control muscles of the same animals showed only normal variation.

Degenerative Changes.—Two types of degeneration were observed. One appeared to follow the extreme light and dark banding in which portions of the fiber were stretched (as in fig. 1 *F*, fiber *a*). Here the dark portions became granular and the light parts disintegrated (as in fig. 1 *G* fiber *b* and fig. 2 *C* fiber *a*). There sometimes was and sometimes was not an aggregation of nuclei about these granular areas, but when there was, most of them were found in the light regions at the edges of the dark granular areas. In the stage preceding granulation of the deeply staining parts of the fiber (fig. 2 *C* fibers *b*, *c* and *d*), muscle nuclei seemed to be even less numerous than in normal muscle. This type of degeneration was not seen in animals earlier than seven days after injection of the toxin. The other type of degeneration was found in muscles that reached the state of permanent contracture as early as five days from the time of injection, as well as in later stages, but it never involved more than a small percentage of the fibers. This degeneration was characterized by an aggregation of a large number of nuclei within a fiber, some of which appeared to be muscle nuclei in greater numbers than normal, and some, large mononuclear cells. Such an aggregation may be found throughout the greater part of a single fiber or only in a small portion of a fiber, as shown in *a* in figure 2 *B*. Serial sections of such a fiber showed that the nuclei were within the fiber and not merely on the surface. In some places, the muscle fiber

10. Jordan, H. E.: Am. J. Anat. **27**:1, 1920.

seemed to have been entirely replaced by such nuclei, which filled the tube formed by the sarcolemma. Wisbaum [6] noted a similar degeneration of single fibers in specimens of tetanic muscles. In such fibers, he observed an increase in the number of muscle nuclei, which in the process of degeneration were only slightly different in appearance from large mononuclear cells which he had seen in hemorrhagic areas between fibers. Stangl [5] injected a suspension of tetanus bacilli into the thighs of guinea-pigs. In the earlier stages, within twelve hours after the injection, there was a local reaction defined by cellular invasion in which polymorphonuclear leukocytes predominated. A few mononuclear leukocytes and large mononuclear wandering cells were also present. The nuclei of the sarcolemma were swollen. Five or six days later, waxy degeneration, disappearance of striations and disintegration of nuclei were seen in muscle fibers in the immediate vicinity of the proliferating tetanus bacilli. Forbus [7] described waxy degeneration in scattered fibers of the rectus abdominis muscles in patients suffering from pneumonia. A swelling of the fiber was followed by proliferation of muscle nuclei, loss of striation and disintegration of the contractile substance. The fiber was then invaded by large mononuclear phagocytic cells. These changes sometimes occurred in single fibers or even in parts of fibers. They were observed in cases of only four days' duration of the disease. In experimentally produced degeneration, he [11] demonstrated by vital staining that the phagocytic cells originated outside the fiber. Although the aggregations of nuclei that we have observed in a few fibers in tetanus appeared similar to those which he described, we did not observe the swelling of individual fibers preceding such an increase in the number of nuclei.

While an increase in the number of muscle nuclei was apparent in many fibers of tetanic muscle, still, regions could be found where an increase could not be seen, as shown in portions of figure *2 C*. This might have been expected, since the other effects of the toxin were not uniform throughout the muscle. Two kinds of nuclei were observed in the fibers of both normal and tetanic muscles, a long, oval type and a large, rounded type, both staining lightly with hematoxylin. Where an increase in number was noted, it concerned both kinds, the long oval nuclei frequently being found in chains of from three to five or more, or in groups closely packed together; the round ones in groups of from five to seven, but with the individual nuclei slightly separated from one another. Fragmented nuclei were also sometimes seen, especially in regions in which degeneration was more advanced. Nuclei of the oval type seemed slightly swollen in the tetanic muscles.

11. Forbus (footnote 7, p. 486).

No increase in either intrafasicular or interfascicular connective tissue was found in any of the preparations.

Vacuoles were observed in muscles five, seven and eight days after the injection of the toxin, though not all specimens showed them. These were best seen in cross-sections, but if there was a greater degree of vacuolation, they were apparent in longitudinal sections also, the fibrils being widely separated in parts of the fiber. In a number of fibers in which we noted vacuolation in cross-sections, we saw nuclei in the interior of the fiber near the edges of the vacuoles. Auriat [12] described fibers with widely separated fibrils having clear spaces between them in preparations of muscles in which experimental edema had been produced. The vacuoles in our preparations presented such an appearance, though in the gross the muscles did not seem edematous. When the toxin of tetanus was injected into the muscles on the back of the thigh, little edema of the leg was seen even in advanced local tetanus. To determine the extent of edema in the muscles, the right and the left gastrocnemii of five rats with the contracture of tetanus were weighed after fixation in a diluted solution of formaldehyde U.S.P. (1:10) and placed in a desiccator. They were again weighed after five days, seven days and two weeks of drying. Both the tetanic and the control muscles lost water at the same rate, and the final weighing showed that the tetanic muscles had been reduced to 19.8 per cent and the control muscles to 20.3 per cent of their original weight. This difference of 0.5 per cent between the dry weights of the muscles of the two sides seems insignificant to us.

The changes found in the gastrocnemius muscle were present to an even more marked extent in the soleus muscle.

SUMMARY

In advanced stages of local tetanus, the extensor muscles fail to relax after section of the motor nerves. This myostatic contracture was studied histologically in the gastrocnemius and soleus muscles of white rats. There is no increase in connective tissue and the contracture is not due to fibrosis. The muscle fibers undergo changes, but what relation these changes bear to the shortening of the muscle is not obvious.

The muscle fibers show a blurring of the cross striations and a mottled staining. The blurring of the cross striations appears to be due, in part, to a disruption of whatever holds the myofibrils in close juxtaposition and accurate transverse alinement. The fibers acquire a wavy longitudinal striation due to the greater evidence of the individual fibrils. The mottling is due to changes in staining reaction of different

12. Auriat, G.: Compt. rend. Soc. de biol. **97**:73, 1927.

parts of the same fiber, some areas staining more heavily, others more lightly than normal.

In some instances the lightly stained areas represent stretched portions of the muscle fibers. Here the ratio of the widths of the *J* bands to the *Q* bands is high, owing to the widening of the *J* bands. In other areas, the ratio is normal, and in some it appears to be slightly reduced as a result of the narrowing of the *J* bands.

A small percentage of the muscle fibers undergo degeneration. Where the separation into light and dark areas has been pronounced, the light fields may disintegrate while the dark ones become granular. A few other fibers become replaced either for short distances or throughout their lengths by nuclei, which fill the old sarcolemma. These appear to be derived, in part, from the nuclei of the muscle and, in part, from mononuclear cells that have invaded the fibers from without.

A HISTOLOGIC STUDY OF THE FORMATION OF BILE PIGMENT *

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The purpose of this report is to present histologic evidence bearing on the origin and on the site of the formation of bile pigment. An endeavor has been made to demonstrate in the various tissues of the body the splitting up of hemoglobin into bile pigment and free iron. Recent articles by Mann,[1] Rich,[2] M'Nee[3] and Whipple[4] are excellent reviews on this subject. Aschoff,[5] Oberling[6] and Krumbhaar[7] have summarized present views concerning the reticulo-endothelial system.

One of the first observations of the extrahepatic formation of bile pigment was made by Virchow[8] in 1847. He found a substance which he called hematoidin occurring in the tissues around old hemorrhages discovered at necropsy. The hematoidin, which occurs both intracellularly and lying free in the tissues, is identical in all its physical and chemical properties with bilirubin, as has been shown recently by Rich and Bumstead.[9] Hooper and Whipple[10] discovered bilirubin on the day following the introduction of a solution of hemoglobin into the

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1. Mann, F. C.: The Site of Formation and Source of Bilirubin, Arch. Path. **2**:516 (Oct.) 1926.

2. Rich, A. R.: The Formation of Bile Pigment, Physiol. Rev. **5**:182, 1925.

3. M'Nee, J. W.: Jaundice; a Review of Recent Work, Quart. J. Med. **16**:390, 1922-1923.

4. Whipple, G. H.: The Origin and Significance of the Constituents of the Bile, Physiol. Rev. **2**:440, 1922.

5. Aschoff, Ludwig: Lectures on Pathology, New York, Paul B. Hoeber, 1924.

6. Oberling, C.: Le système reticulo-endothelial, Ann. d'anat. path. **1**:87, 1924.

7. Krumbhaar, E. B.: The So-Called Reticulo-Endothelial System, Its Relation to Phagocytosis Defense Processes; Lipoid and Protein Metabolism; Destruction of Red Cells and to Neoplasms, Internat. Clin. **2**:280 (35th s.) 1925.

8. Virchow, R.: Die pathologischen Pigment, Virchows Arch. f. path. Anat. **1**:379, 1847.

9. Rich, A. R., and Bumstead, J. H.: On the Identity of Haematoidin and Bilirubin, Bull. Johns Hopkins Hosp. **36**:225, 1925.

10. Hooper, C. W., and Whipple, G. H.: Icterus: A Rapid Change of Hemoglobin to Bile Pigment in the Pleural and Peritoneal Cavities, J. Exper. Med. **23**: 137, 1916.

pleural and peritoneal cavities of dogs. Van den Bergh,[11] using his quantitative method, demonstrated a high content of bilirubin in old hematomas. The conversion of hemoglobin into bile pigment in subdural hematomas was discussed recently by Putnam and Cushing.[12]

That hemoglobin is one source, if not the only source, of bilirubin is indicated by a consideration of the probable formulas of these substances, and of the intermediary products. The generally accepted view (Mathews [13]) is that hemoglobin splits up into a hematin and a globin fraction. The hematin liberates iron and becomes hematoporphyrin, which is isomeric with bilirubin, the reaction being represented by the equation:

$$\underset{\text{hematin}}{C_{32}H_{32}N_4O_4Fe} + 2H_2O = Fe + \underset{\text{hematoporphyrin or bilirubin}}{C_{32}H_{36}N_4O_6}$$

Biliverdin is formed by the oxidation of bilirubin. The excessive formation of bilirubin, as shown by jaundice and bilirubinuria, in clinical conditions involving the massive destruction of blood gives further evidence in favor of the derivation of bilirubin from hemoglobin. Such conditions include hemolytic icterus, pernicious anemia and paroxysmal hemoglobinuria.

The problem with which this paper is chiefly concerned is that of the site of the formation of bilirubin. As early as 1769, Morgagni [14] taught that the liver excreted the bile which was brought to it, preformed, by the blood. An altogether different view was generally accepted for many years following the experiments of Minkowski and Naunyn,[15] in 1886, who found that jaundice was produced in geese by poisoning with arseniuretted hydrogen, whereas jaundice could not be so produced if the liver was removed immediately after the administration of this substance. From this they concluded that the liver was necessary for the production of jaundice, and the belief arose that bile pigment was formed by the hepatic cell.

The aforementioned experiments were repeated by M'Nee,[16] who found that small traces of bile appeared in the urine and the tissues

11. Van den Bergh, A. A. H.: Der Gallenfarbstoff im Blut, Leiden, Van Doesburgh, 1918.

12. Putnam, T. J., and Cushing, Harvey: Chronic Subdural Hematoma; Its Pathology; Its Relation to Pachymeningitis Hemorrhagica and Its Surgical Treatment, Arch. Surg. **11**:329 (Sept.) 1925.

13. Mathews, A. P.: Physiological Chemistry, ed. 4, New York, William Wood & Company, 1925.

14. Morgagni, quoted by Rich (footnote 2).

15. Minkowski, O., and Naunyn, B.: Beiträge zur Pathologie der Leber und des Icterus. Ueber den Icterus durch Polycholie und die Vorgänge in der Leber bei demselben, Arch. f. exper. Path. u. Pharmakol. **21**:1, 1886.

16. M'Nee, J. W.: Experiments on Haemolytic Icterus, J. Path. & Bact. **18**:325, 1913-1914.

of hepatectomized geese following the administration of arseniuretted hydrogen. M'Nee observed, in histologic studies of the normal goose's liver, that the Kupffer cells contained erythrocytes and gave a marked iron reaction. In the livers of jaundiced geese, the Kupffer cells were seen to contain, besides many erythrocytes, large quantities of yellowish-green pigment, which resembled biliverdin, although chemical proof was lacking. McNee concluded that, in geese, bile pigment was formed by the endothelial (Kupffer) cells of the liver and by the small number of endothelial cells found elsewhere (spleen and bone-marrow). In geese, hepatectomy accomplishes the removal of the greater part of the reticulo-endothelial system, but in mammals it does not.

Before reviewing further experimental work, one should consider the type of cell of which the Kupffer cell is an example. During the last half century, certain fixed cells and certain wandering cells having phagocytic properties have been studied by many workers. Ranvier [17] gave the name "clasmatocytes" to phagocytic cells found in the connective tissues. Mechnikov [18] divided phagocytes into two classes: microphages or polymorphonuclear leukocytes and fixed tissue macrophages, which included the large cells of the splenic pulp and lymph nodes, the Kupffer cells of the liver, the neuroglia and nerve cells and certain cells of the connective tissues. Ribbert,[19] in 1904, showed the interrelation of all these different cells in their common ability to engulf particles of lithium carmine injected into the circulation. The marked phagocytic activity of the stellate (Kupffer) cells for particulate matter was demonstrated in a recent paper by Higgins and Murphy.[20]

To Aschoff belongs the credit for the conception that these reticular and endothelial cells of the various tissues possess common properties and functions, and may be designated all together as the reticulo-endothelial system. He divided the cells of this system into two groups: reticulo-endothelial cells and histiocytes. In the first group, he included reticular cells of the spleen, bone-marrow and lymphatic tissue, and endothelial cells of the liver, lymph sinuses, splenic sinuses, bone-marrow, suprarenal capillaries and hypophyseal capillaries. To the second group, he assigned tissue histiocytes (clasmatocytes), splenocytes and blood histiocytes (endothelial leukocytes).

Many attempts have been made to determine what tissues are most active in the conversion of hemoglobin into bile pigment. The forma-

17. Ranvier, L.: Des clasmatocytes, Arch. d'anat. micr. **3**:122, 1900.

18. Mechnikov, E.: Immunity in Infective Diseases, Cambridge, Harvard University Press, 1905.

19. Ribbert, Hugo: Die Abscheidung intravenös injizierten gelösten Karmins in den Geweben, Ztschr. f. allg. Physiol. **4**:201, 1904.

20. Higgins, G. M., and Murphy, G. T.: The Phagocytic Cells (v. Kupffer) in the Liver of Common Laboratory Animals, Anat. Rec. **40**:15, 1928.

tion of bilirubin in the spleen has been proved by measurements of the bilirubin content of the splenic vein and artery. Van den Bergh and Snapper [21] reported that in five of six cases of hemolytic anemia the blood of the splenic vein contained more bilirubin than did that of the peripheral veins. Similarly, Rich and Rienhoff [22] found an increased amount of bilirubin in the blood of the splenic vein compared with that of the splenic artery and the peripheral veins in four of ten cases representing various pathologic conditions. In these two studies, the quantitative estimation of the bilirubin was made with the van den Bergh method. The spectrophotometric determination of the bilirubin content of the blood in vessels issuing from the spleen and from the bone-marrow showed, in the work of Mann, Bollman, Sheard and Baldes,[23] that more bilirubin was normally present in these vessels than in the arteries to the spleen and the bone-marrow. From a continuation of these studies with removal of the spleen or the liver or all abdominal viscera, they [24] concluded: "While bilirubin is made in both the spleen and the liver the amount is insignificant as compared with that made in the bone-marrow."

The experimental work on the site of the formation of bile pigment has been directed largely toward the proof of such formation after the removal of the liver. The earliest work of this kind was done by Müller,[25] in 1844, on frogs, and later by Kunde [26] and by Moleschott.[27] All of these experimenters removed the livers of the frogs, and in no case did they observe thereafter the formation of bile pigment. The experiments of Minkowski and Naunyn on geese have been described. The earliest efforts to exclude the liver in mammals were those of Whipple and Hooper,[28] who ligated the principal blood vessels to the liver in dogs, and found that the subsequent intravenous injection of hemoglobin was followed by the development of jaundice in the tissues.

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21. Van den Bergh, A. A. H., and Snapper, I.: Ueber anhepatische Gallenfarbstoffbildung, Berl. klin. Wchnschr. **2**:1081, 1915.

22. Rich, A. R., and Rienhoff, W. F., Jr.: The Bile-Pigment Content of the Splenic Vein, Bull. Johns Hopkins Hosp. **36**:431, 1925.

23. Mann, F. C.; Bollman, J. L.; Sheard, C., and Baldes, E. J.: The Site of the Formation of Bilirubin, Am. J. Physiol. **74**:497, 1925.

24. Mann, F. C.; Sheard, Charles; and Bollman, J. L.: An Evaluation of the Relative Amounts of Bilirubin Formed in the Liver, Spleen and Bone Marrow, Am. J. Physiol. **78**:384, 1926.

25. Müller, J.: Handbuch der Physiologie des Menschen für Vorlesungen, Coblenz, J. Hölscher, 1840-1844, vol. 4, p. 131.

26. Kunde, F. T.: De hepatis ranarum extirpatione, Berlin, Schlesinger, 1850.

27. Moleschott, J.: Untersuchungen über die Bildungsstätte der Galle, Arch. f. physiol. Heilk. **11**:479, 1852.

28. Whipple, G. H., and Hooper, C. W.: Hematogenous and Obstructive Icterus. Experimental Studies by Means of the Eck Fistula, J. Exper. Med. **17**: 593, 1913.

A new operative method which made it possible for dogs to survive total hepatectomy for several hours was devised by Mann and Magath.[29] The operation was performed in three stages: (1) production of a reverse Eck fistula, (2) ligation of the portal vein and (3) complete removal of the liver together with a segment of the inferior vena cava. The animals were kept alive for from fifteen to thirty hours by the intravenous injection of a solution of dextrose. Bile pigment appeared in the blood within from three to six hours after the hepatectomy, and its appearance could be hastened by the intravenous injection of hemoglobin. Even the removal of all abdominal viscera failed to prevent the formation of bilirubin.

Rich [30] noted striking results in the application of the method of tissue culture to this problem. He added fresh erythrocytes to coverglass cultures containing wandering phagocytes of mesodermal origin. These phagocytes were seen to ingest large numbers of erythrocytes, and as the hemoglobin of the latter was broken down, crystals of bilirubin and biliverdin appeared within the phagocytes. The identity of these pigments was established by the microscopic Gmelin test and by their structure and color. The Berlin blue reaction often demonstrated an iron-containing residue in these phagocytes by the side of the bile pigment.

A few instances have been reported of the conversion of hemoglobin into bilirubin in the test tube, when such agents as trypsin and dextrose or pyogenic bacteria were employed. Careful repetition of these experiments by Rich and Bumstead [31] failed to show the presence of bilirubin by the van den Bergh test and by extraction with chloroform.

Since the results reported in this paper depend largely on the occurrence of inorganic iron in certain tissues, it seems advisable to summarize briefly the knowledge of the metabolism and distribution of iron in the body. Asher [32] believed that the spleen stands first among the regulators of iron metabolism. If this organ is removed, the excretion of iron through the intestines is increased, and anemia may result, unless this is prevented by the compensatory functioning of other

29. Mann, F. C., and Magath, T. B.: The Effect of Total Removal of the Liver, Tr. Sect. Path. and Physiol., A. M. A., 1921, p. 29.

30. Rich, A. R.: The Formation of Bile Pigment from Haemoglobin in Tissue Cultures, Bull. Johns Hopkins Hosp. **35**:415, 1924.

31. Rich, A. R., and Bumstead, J. H.: On the Alleged Power of Bacteria to Form Bile Pigment from Haemoglobin, Bull. Johns Hopkins Hosp. **36**:376, 1925; On the Question of the Formation of Bile Pigment from Haemoglobin by the Action on Enzymes, Bull. Johns Hopkins Hosp. **36**:437, 1925.

32. Asher, Leon: Ueber die Bedingungen der Blutbindung und des Eisenstoffwechsels, Med. Klin. **21**:1909, 1925.

organs. The other reticulo-endothelial cells are also concerned in iron metabolism.

A microscopic study of the occurrence of iron in the spleen as compared with that in the liver in domestic animals was recently made by Ziegler and Wolf.[33] They found much iron in the spleen of the horse, cow and sheep and a smaller amount in the spleen of the dog and swine. The iron occurred chiefly in the reticular cells of the spleen, although traces were seen in the capsule and the trabeculae. Small amounts of iron were sometimes found in the livers of all the species studied. The iron, when found in the liver, was always limited to the endothelial (Kupffer) cells. The bone-marrow was not studied.

Interesting observations were made by Peabody and Broun[34] on the phagocytosis of erythrocytes in the bone-marrow. The phagocytic cells probably should be classified as "endothelial leukocytes," although whether they arise from the vascular endothelium or from the reticulum has not been proved. These workers, employing the Berlin blue iron reaction, observed in the bone-marrow the presence of erythrocytes and granules of iron in the same phagocyte. They concluded that the phagocyte of the bone-marrow ingests erythrocytes and that the hemosiderin (an iron-containing substance) is formed from hemoglobin within the phagocyte.

Corr[35] recently studied material from seventy-one necropsies with respect to the distribution of hemosiderin and bilirubin. Sections of liver and spleen and occasionally of bone-marrow, kidney, lymph node, lung, suprarenal gland and pancreas were stained for iron by the Berlin blue method. Hemosiderin was found in varying amounts in the splenic phagocytes, in the hepatic and Kupffer cells of the liver and in the reticulo-endothelial cells of the bone-marrow. Corr concluded that the reticulo-endothelial cells of the spleen and the bone-marrow were most active in breaking down the hemoglobin. He believed that the Kupffer cells and large wandering phagocytes served as a reserve of the reticulo-endothelial system.

TECHNIC

Dogs and rabbits were used in the preliminary experiments. It was found that in rabbits, when jaundice had been produced experimentally by ligation of the common bile duct and cholecystectomy, the tissues frequently contained bile pig-

33. Ziegler, M., and Wolf, E.: Histochemische Untersuchungen über das Vorkommen eisenhaltigen Pigments (Hämosiderins) in der Milz und Leber der Haussäugetiere unter normalen und einigen pathologischen Verhältnissen, Virchows Arch. f. path. Anat. **249**:374, 1924.

34. Peabody, F. W., and Broun, G. O.: Phagocytosis of Erythrocytes in the Bone Marrow, with Special Reference to Pernicious Anemia, Am. J. Path. **1**:169, 1925.

35. Corr, Philip: Histochemical Evidence Concerning the site of the Formation of Bile Pigment, Arch. Path. **7**:84 (Jan.) 1929.

ment, but that in dogs, when similar experiments were made, bile pigment could not be demonstrated in the tissues. Nevertheless, dogs were used almost exclusively in the later experiments because they survived more extensive surgical procedures than did rabbits. They were the subjects of most of the experiments on the formation of bilirubin in mammals.

Zenker's fluid was found to be the best fixative for general use, although Bouin's fluid showed bile pigment in the tissues of jaundiced rabbits more clearly than did any other fixative. A diluted solution of formaldehyde U. S. P. (1:10) was slightly inferior to Bouin's fluid. Parts of all tissues taken were fixed in Zenker's fluid, and in more than half of the experiments similar pieces of tissue were also fixed in Bouin's fluid or formaldehyde. Such tissues were embedded in paraffin and from them sections 7.5 microns thick were cut.

Another method was to macerate the fresh tissues in Pacini's fluid consisting of 5 per cent mercuric chloride in physiologic solution of sodium chloride. With this method, Löwit[36] had been able to demonstrate bile pigment by the microscopic Gmelin reaction in the hepatic cells of frogs. These results could not be duplicated in the tissues of dogs, except in the case of bile plugs filling the bile capillaries, which showed the typical play of colors with the Gmelin reagent in paraffin sections of livers from jaundiced dogs. Frozen sections of formaldehyde-fixed material were also studied with the Gmelin reagent and various stains. These methods, which rendered the use of alcohol unnecessary, eliminated the possibility that bilirubin was being extracted from the tissues by the alcohol used in dehydration.

Various combinations of stains were employed in the hope of demonstrating bilirubin or other products of hemoglobin disintegration in the tissues. Of the nonspecific stains, the combination of eosin and methylene blue (Methylthionine chloride, U. S. P.), as described by Mallory and Wright,[37] gave a striking demonstration of a yellowish-brown pigment occurring normally in the spleen and the bone-marrow. This pigment was barely distinguishable in sections stained by the hematoxylin and eosin method, which has such widespread use. Mallory's triple connective tissue stain (acid-fuchsin, orange G and aniline blue) also showed these masses of pigment, although the cytologic details were not clear.

One of the products of hemoglobin disintegration has long been known to give a specific reaction for iron. Three microchemical specific iron stains are in common use: ammonium sulphide, potassium ferrocyanide and hydrochloric acid (the Berlin blue or Prussian blue reaction), and potassium ferricyanide and hydrochloric acid (Turnbull's blue). After many trials, the Berlin blue method was selected, because it gave a characteristic deep blue color to any iron present in the tissues. Various counterstains can be used with these iron reagents. Basic fuchsin, the usual stain employed with potassium ferrocyanide, was abandoned in favor of alum carmine, because the faint red nuclear stain given by the latter provided a better contrast to the deep blue of the iron-containing pigment.

The method finally adopted was as follows: Sections of tissues fixed in Zenker's fluid, formaldehyde or Bouin's fluid were treated with potassium ferrocyanide and hydrochloric acid for thirty seconds at 70 C., as described by Mallory and Wright. The sections were then washed and counterstained for twenty minutes in alum carmine warmed to 50 C. In this way, iron was stained a dark

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36. Löwit, M.: Beiträge zur Lehre vom Icterus, Beitr. z. path. Anat. u. z. allg. Path. **4**:223, 1889.

37. Mallory, F. B., and Wright, J. H.: Pathological Technique, ed. 8, Philadelphia, W. B. Saunders Company, 1924.

or light blue depending on its concentration in the cell, the nuclei were stained red and the cytoplasm, in general, a faint pink. Erythrocytes were stained a faint yellowish green.

IRON-CONTAINING PIGMENTS AS SEEN NORMALLY IN BONE-MARROW, SPLEEN AND LIVER

Before the various experiments are described, it is advisable to present the picture normally seen in the bone-marrow, spleen and liver. Such tissues were embedded in paraffin and stained with Berlin blue and alum carmine to demonstrate the occurrence of iron-containing pigment. In the bone-marrow (fig. 1), this pigment was seen in the form of dark

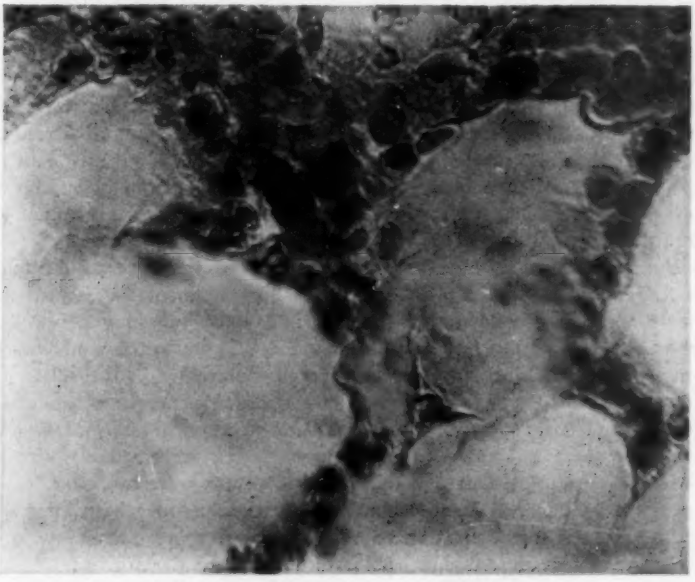

Fig. 1.—Bone-marrow before injection of hemoglobin; × 1,000. The section shown here and in subsequent figures were all stained for iron by the Berlin blue method.

masses which were actually blue in the section, but which photographed black with a red screen. The masses lay as single droplets or clusters of droplets in large cells of reticular type, which were seen between fat droplets and mingled with the other cellular elements of the bone-marrow. Such cells often showed a diffuse faintly blue stain in addition to the dark blue droplets.

A section of the spleen likewise showed large numbers of the dark masses, which were found in the large phagocytic cells usually called splenic cells. A diffuse faintly blue stain also was seen in some of these cells. None of the iron-containing pigment was found in the malpighian corpuscles.

A wide variation in the iron content of the liver was found in normal dogs. The iron-containing pigments appeared solely in the stellate (Kupffer) cells of the sinusoids of the liver. The pigment usually occurred in the form of clustered droplets, and a faintly blue stain was found in many of the stellate cells. An iron-containing pigment was not seen in any of the parenchymal, or hepatic, cells. Small amounts of iron were found in the various lymph nodes, as will be discussed later.

EXPERIMENTS

In the first series of experiments, the dogs were anesthetized and pieces of liver, spleen, bone-marrow, lymph nodes and various other tissues were removed. The hemolyzed corpuscles from 100 cc. of dog's blood were injected into the jugular vein. At intervals of from fifteen minutes to three hours thereafter, additional pieces of the same organs were excised. Samples of blood from the jugular vein, corresponding in time to the various sets of tissues removed, were studied by the spectrophotometric method devised by Sheard, Baldes, Mann and Bollman,[38] to determine the quantity of bilirubin in the circulating blood at the various stages of the experiment. The protocols of three typical experiments are given.

EXPERIMENT 1.—A dog weighing 27.3 Kg. was anesthetized with ether at 3:05 p. m. and pieces of the liver, spleen, bone-marrow (right femur) and mesenteric lymph node were removed. Fifteen cubic centimeters of blood was withdrawn from the jugular vein for the determination of bilirubin. At 3:10 p. m., the laked corpuscles from 100 cc. of the blood of another dog were injected into the jugular vein. This solution was made isotonic by the addition of sodium chloride and was warmed to 37 C. At 3:25 p. m., a piece of bone-marrow (right humerus) was removed. At 3:40 p. m. pieces of liver, spleen and bone-marrow (left femur) were removed, and 15 cc. of blood was withdrawn from the jugular vein. At 4:10 p. m., pieces of liver, spleen, bone-marrow (left humerus), retrosternal lymph node and mesenteric lymph node were removed and 15 cc. of blood was withdrawn from the jugular vein. The animal was killed. The results of the postmortem examination were negative.

Spectrophotometric studies of the samples of blood, by the method described by Sheard, Baldes, Mann and Bollman, showed a marked increase in bilirubin content during the course of the experiment. This was indicated by increased absorption in the region of wave length 500 to wave length 430, due to the bilirubin in the acetone-alcohol solution of the blood sample. At wave length 430, the first sample showed 75 per cent transmission, the second showed 51 per cent and the last showed 50 per cent.

The tissues removed during the experiment were fixed in Zenker's fluid, sectioned in paraffin and stained by the Berlin blue method. A study of these sections did not show any recognizable change in the quantity of iron-containing pigment in the liver, spleen and mesenteric lymph nodes. A section of the bone-marrow removed before the injection of hemoglobin contained a small amount of iron. A similar section taken one hour after the injection of the hemoglobin had more than twice as much of the iron-containing pigment. This increase occurred chiefly in the form of tiny greenish-blue droplets, or clusters of droplets, which were

38. Sheard, Charles; Baldes, E. J.; Mann, F. C., and Bollman, J. L.: Spectrophotometric Determinations of Bilirubin, Am. J. Pysiol. **76**:577, 1926.

always found lying within reticular cells of the bone-marrow. When these tissues were studied unstained, or stained by the eosin and methylene-blue method, the intracellular droplets were an orange-yellow, and were the only pigments appearing in the sections.

EXPERIMENT 2.—This experiment was similar to experiment 1, although covering a slightly longer period of time. A dog weighing 22 Kg. was etherized, and pieces of liver, spleen and bone-marrow were removed, after which the laked corpuscles from 100 cc. of dog's blood were injected into the jugular vein. Additional pieces of the same organs were excised at intervals of thirty minutes, one hour, two hours and three hours after the injection of laked blood. At the same time, blood was withdrawn for the determination of the serum bilirubin.

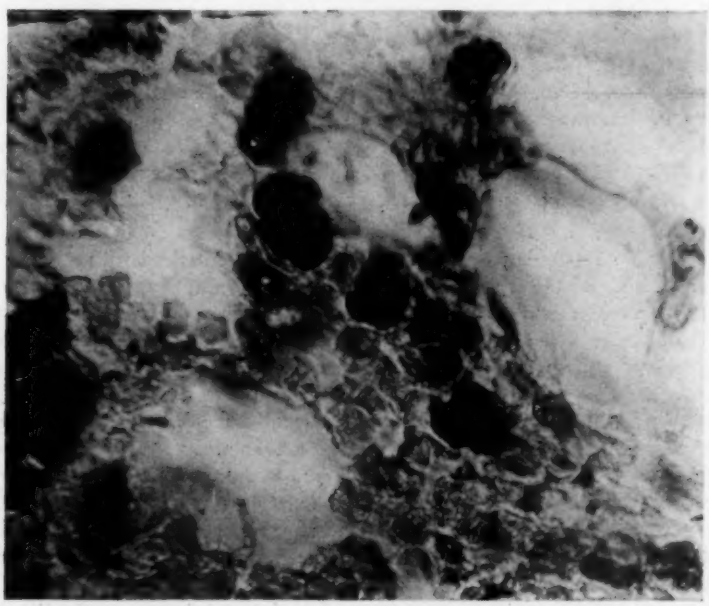

Fig. 2.—Bone-marrow thirty minutes after injection of hemoglobin; × 1,000.

Examination, by the Berlin blue method, of the tissues removed in the course of the experiment showed a moderate number of masses of iron-containing pigment lying in reticular cells of the bone-marrow before the injection of hemoglobin (fig. 1). The specimen of bone-marrow removed thirty minutes after the injection of hemoglobin contained about three times as much of this pigment, which appeared as grapelike clusters of greenish-blue droplets and was intracellular (fig. 2). Figure 3 shows the condition of the bone marrow two hours after the hemoglobin was injected. Here about twice as much iron-containing pigment was seen as at the beginning of the experiment. Bone-marrow removed after three hours also showed a marked increase in the pigment.

The liver, prior to the injection of hemoglobin, showed small amounts of iron confined almost entirely to the tissues of the periportal spaces. The amount of iron pigment increased slightly two hours after the hemoglobin was injected, and the increase was more marked after three hours. Then small quantities of iron could be seen in occasional stellate cells, and more in the periportal spaces.

The spleen contained a moderate amount of iron before the injection of the hemoglobin, and a noticeable increase did not occur during the course of the experiment. The samples of blood from the jugular vein were studied by the spectrophotometric method. A marked increase in the bilirubin in the serum was shown by the percentage of transmission at wave length 430; this was 87 before the injection of laked blood and 57, 59 and 64 in the samples withdrawn following its injection.

In two experiments the animals were allowed to live six hours or longer after the injection of hemoglobin.

EXPERIMENT 3.—A dog weighing 13.2 Kg. was etherized and pieces of liver, spleen and bone-marrow were removed before and one hour after the intravenous

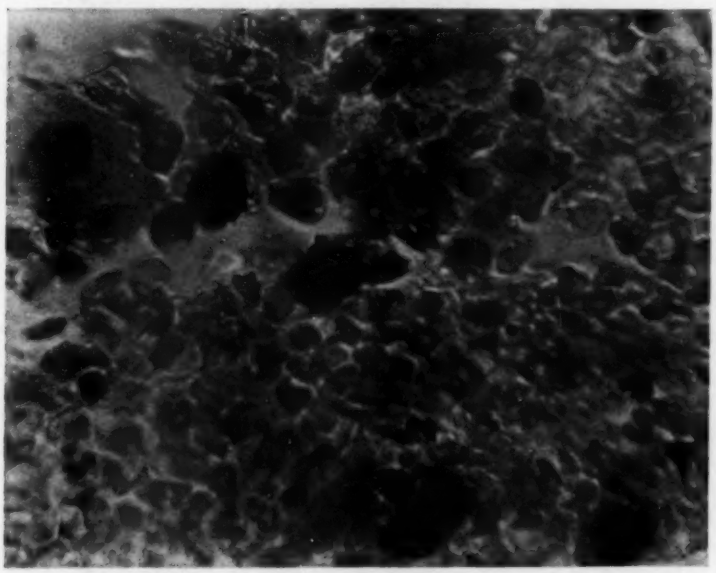

Fig. 3.—Bone-marrow two hours after injection of hemoglobin; × 1,000.

injection of laked corpuscles. The incisions were then closed. Five hours later the animal appeared to be in good condition, but died during the night. The results of the necropsy twenty-two hours after the injection of laked blood were negative, except for the presence of about 30 cc. of thick bloody fluid in the peritoneal cavity. The usual tissues were removed for study.

Under the microscope in the first series, a small amount of iron-containing pigment was seen in the liver and the mesenteric lymph node, and comparatively small quantities in the spleen and the bone-marrow (fig. 4). In the other two series there was little, if any, change in the liver, spleen and lymph nodes, while the iron content of the bone-marrow was about doubled. In the bone-marrow (fig. 5), one hour after hemoglobin was injected, there was seen to be a marked increase in the number of greenish-blue droplets, which appeared to be the form that the hemoglobin assumes when it is first taken up by the reticular cells. The bone-marrow (fig. 6), from six to twenty-two hours after the injection of hemo-

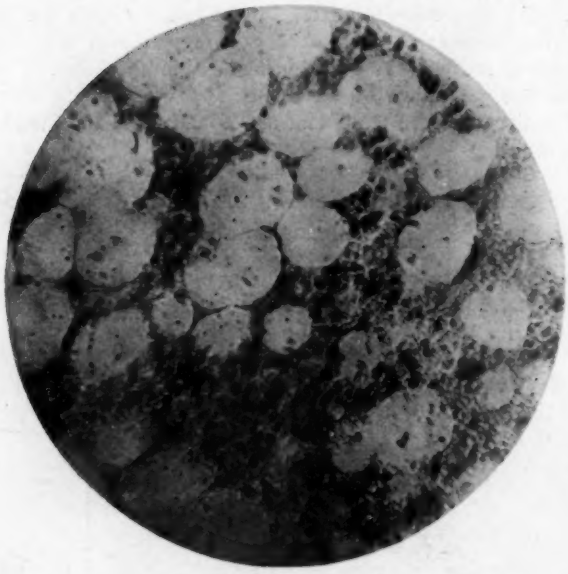

Fig. 4.—Bone-marrow before injection of hemoglobin; × 200.

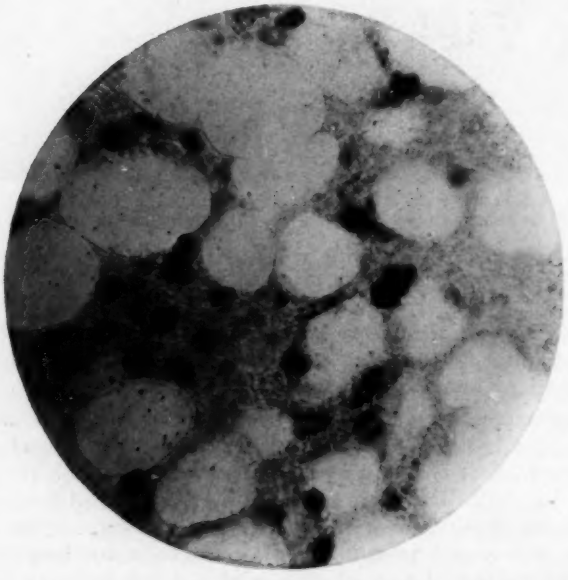

Fig. 5.—Bone-marrow one hour after injection of hemoglobin; × 200.

globin, contained similar droplets which stained a deeper blue, suggesting that more free iron had been liberated from engulfed hemoglobin. Such deep blue droplets were seen in most sections of normal bone-marrow.

Determinations of the serum bilirubin showed marked increase in bile pigment in the circulating blood after one hour, and a less degree of bilirubinemia after from six to twenty-two hours. At wave length 430, the first sample gave 85 per cent transmission, the second gave 20 per cent transmission and the last 51 per cent transmission.

Experiments 1, 2 and 3 were selected from a group of nine because in them the results were most striking. In three other experiments there was a definite, although moderate, increase in the amount of iron-

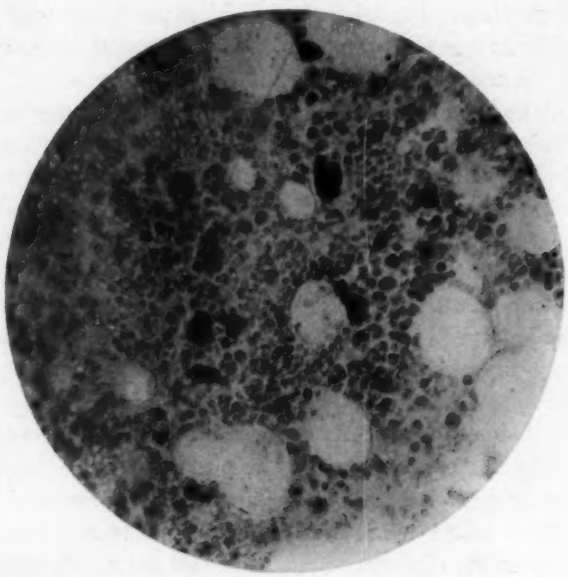

Fig. 6.—Bone-marrow from six to twenty-two hours after injection of hemoglobin; × 200.

containing pigment in the reticular cells of the bone-marrow; in the remaining three experiments, a difference could not be detected between the bone-marrow before and that after the injection of hemoglobin. With the exception of that occurring in the first experiment, there was little, if any, change in the iron content of the liver. As a rule, the picture in the spleen remained the same after the injection of hemoglobin as before, although in one experiment an apparent slight increase in iron was noted. Since considerable amounts of iron are usually scattered diffusely through the splenic pulp, it is impossible to say with certainty from a microscopic study that an increase in the iron content did not occur.

When one considers the wide distribution of the bone-marrow, it is not surprising that a third of the experiments failed to show an increased content of iron following the injection of hemoglobin. Only a tiny fragment of marrow was examined and one can merely guess at the changes going on in the remainder of the skeleton. It is significant, however, that in none of the cases did sections of bone-marrow taken after such an injection show a decrease in iron content. One is justified, therefore, in attributing the increased iron content of the bone-marrow in the cited cases to the injection of hemoglobin. This substance is taken up by the reticular cells of the bone-marrow, in which it breaks down, with the liberation of iron and the formation of bilirubin or an intermediary product. Probably the bilirubin passes into the circulating blood as rapidly as it is elaborated and, for this reason, is never deposited in the tissues in a demonstrable form.

Two experiments were carried out in much the same way as those described except that the 100 cc. of laked blood was injected into a tributary of the portal vein instead of into the jugular vein, thus forcing the hemoglobin to pass through the liver before it reached the bone-marrow and the spleen. In both experiments, the blood serum showed a high degree of bilirubinemia after from one to two hours. A slight increase in iron content occurred in the liver, without any definite change in the spleen. In one experiment there was a moderate increase of iron-containing droplets in the bone-marrow; a change was not seen in the bone-marrow in the other experiment. The jaundice may have been largely of an obstructive type owing to injury of the hepatic cells by the injection of hemoglobin into the portal vein.

In two other experiments, splenectomy was performed just prior to the injection of laked blood into the jugular vein. In one of the experiments observations for two and a half hours showed a marked rise in serum bilirubin. The tissues showed a slight increase in the iron content of the liver and a slight rise in the number of iron-containing droplets in the bone-marrow. The dog in the second experiment was allowed to live twenty-four hours and was then killed. Before splenectomy and the injection of hemoglobin, the liver contained a small amount of iron. Twenty-four hours later there was a marked increase in the iron-containing pigment in the stellate cells and occasionally in small periportal spaces. The bone-marrow showed a slight increase in iron-containing droplets. From these experiments, it would seem that removal of the spleen may cause the liver to assume a more prominent part than it usually does in the formation of bilirubin, although definite conclusions cannot be drawn from such limited data.

It was thought that a rapid loss of blood might cause a demonstrable decrease in the iron content of various tissues, since the rate of production of hemoglobin would probably be accelerated. Pieces of liver,

spleen and bone-marrow were removed from a dog weighing 15 Kg. Two hundred cubic centimeters of blood was withdrawn from the jugular vein, and additional pieces of the tissues were taken at necropsy following the animal's death from seven to twenty-two hours later. The amount of iron present in the different series of tissues did not show appreciable change. In a similar experiment, 250 cc. of blood was withdrawn from a dog weighing 20 Kg. The tissues taken before hemorrhage were compared with those removed an hour later and with those removed forty-eight hours later. In this case also, the iron content of the liver, spleen and bone-marrow remained the same throughout the experiment.

In order to rule out, as an important factor, the influence of the operative procedure on the organs studied, two experiments were performed in which the pieces of liver, spleen and bone-marrow were removed at from thirty-minute to sixty-minute intervals up to two and a half hours, but in which laked blood was not injected. These tissues did not show definite change as regards iron content, thus indicating that the microscopic changes observed in previous experiments were entirely the result of the injection of hemoglobin into the blood stream.

In the aforementioned protocols of experiments little has been said of the lymph nodes and various other members of the reticulo-endothelial system. Pieces of mesenteric lymph nodes were removed during the course of most of the experiments. The sections invariably showed either small traces of iron or its complete absence, without difference between those pieces removed before and those removed after the injection of hemoglobin.

One or two small nodes were usually found adherent to the deeper surface of the sternum near its upper end. Such nodes were usually reddish brown and contained much blood, sometimes resembling hemolymph nodes and at other times true lymph nodes. In one animal, a blood vessel was demonstrated to be passing into such a node. The Berlin blue stain showed varying amounts of iron, as well as considerable coal dust, present in the medullary portions of these nodes. In many cases, the iron was present in relatively large quantities, although the site of these nodes rendered it impossible to obtain specimens from the same animal before and after hemoglobin had been injected. Bronchial lymph nodes contained moderate amounts of iron.

Other organs, namely, the thymus gland, pituitary gland, suprarenal gland, testis, ovary and kidney, were stained for iron. This substance was invariably absent except for occasional minute traces in the connective tissue of the ovary, where it probably had resulted from the hemorrhage into ruptured graafian follicles. Even after the injection of hemoglobin, the kidney remained free from demonstrable iron.

The experiments of Hooper and Whipple, who injected laked blood or a solution of hemoglobin into the pleural cavities of dogs, were repeated. These authors found bilirubin present in quantities demonstrable by the Huppert-Salkowski test on the day following the injection of hemoglobin. They concluded that the transformation of hemoglobin into bilirubin is accomplished by the endothelium lining the pleural cavity. In the following experiment, their technic was employed.

EXPERIMENT 4.—A dog weighing 7 Kg. was anesthetized at 2 p. m., November 16. Fifty cubic centimeters of blood was taken from another dog; the erythrocytes were washed with physiologic solution of sodium chloride and then laked with distilled water. This solution was diluted with physiologic solution of sodium chloride to make 400 cc. containing 0.6 per cent sodium chloride, which was injected into the right pleural cavity of the anesthetized animal. At 9 a. m., November 17, 60 cc. of dark red fluid was withdrawn from the right pleural cavity and centrifugated, and smears were made of the sediment. The supernatant fluid was divided into three portions. The first portion was examined by the spectrophotometric method, which proved unsatisfactory owing to the large amount of hemoglobin in the solution. A second portion gave a faint (questionable) indirect van den Bergh reaction for bile pigment. The other portion was subjected to the Huppert-Salkowski test, as used by Hooper and Whipple; it gave a faintly positive reaction. At 9 a. m., November 18, 50 cc. of laked erythrocytes in 200 cc. of 0.6 per cent sodium chloride solution was injected into the left pleural cavity. At 9 a. m., November 20, the animal was killed.

Necropsy revealed nothing abnormal except in the thoracic cavity, which did not contain free fluid. A brown fibrinous exudate covered the parietal pleura near both bases and over the diaphragm. A similar exudate and small black particles appeared on the mediastinal tissue between the heart and the diaphragm. Pieces of tissue from various points in the thoracic cavity were fixed, sectioned and stained by the Berlin blue method.

Smears from the aspirated pleural fluid showed large numbers of erythrocytes and polymorphonuclear leukocytes, in which iron could not be demonstrated. Fixed sections of the fibrinous exudate taken at necropsy showed traces of iron in a few cells resembling the large mononuclear leukocytes of the blood. The most significant observation in this experiment was the presence of iron in many connective tissue cells of the mediastinal tissue (fig. 7). Large numbers of stellate cells with large oval nuclei (evidently belonging to the histiocyte or connective tissue macrophage group) were stained a characteristic blue in sharp contrast with the endothelial cells, which were free from any trace of iron. The iron-containing cells lay immediately subjacent to the surface layer of endothelium. A lymph node on the internal surface of the sternum and one on the posterior thoracic wall both contained large amounts of iron. A small quantity of iron was found in a peribronchial lymph node.

The results obtained in this experiment confirm the earlier work of Hooper and Whipple in so far as they show the presence of bile pigment in the pleural cavity following the injection of hemoglobin. However, it appears that instead of the pleural endothelium (or mesothelium) being the tissue concerned, as these authors assumed, the cells of the subendothelial connective tissue converted the hemoglobin into bilirubin.

Studies on the formation of bilirubin in experimentally produced hematomas of the scalp were reported by van den Bergh. Such an experiment was performed for the purpose of studying the iron content of the tissues.

EXPERIMENT 5.—A dog weighing 23.5 Kg. was anesthetized at 10 a. m., November 11, and 100 cc. of blood was withdrawn from the jugular vein into citrate solution and centrifugated, and the cells were washed with physiologic solution of sodium chloride. The cells were diluted with physiologic solution of sodium chloride to make 90 cc., which was injected as deeply as possible into the scalp over the right parietal bone. These steps were carried out with precautions to keep all solutions sterile. At 10 a. m., November 13, an attempt to aspirate

Fig. 7.—Mediastinal tissue four days after the injection of hemoglobin into the pleural cavity. Two layers of pleural endothelium are seen at the periphery of the field. Iron appears in the connective tissue cells; × 200.

fluid from the site of the injection was unsuccessful. One hundred cubic centimeters of laked blood was injected under the scalp over the left parietal bone. At 10 a. m., November 15, fluid could not be aspirated from the sites of injection. The animal was killed.

Small amounts of blood were found at the sites of injection, and considerable blood had gravitated into the cheeks. Pieces of tissue from the scalp were fixed, sectioned and stained by the Berlin blue method. The sections showed iron present in a large number of cells of the histiocyte type which lay in strands of connective tissue between the muscle bundles. The absence of iron from the muscle bundles themselves was apparent. In sections taken from the region of the right parotid gland, to which blood had gravitated, cells of the areolar tissue also were seen to contain iron.

In a similar experiment, the animal was killed sixteen days after laked blood had been injected under the scalp. Here also (fig. 8) considerable quantities of iron could still be seen in the cells of the connective tissue lying between the bundles of muscle. Although in these experiments it was not possible to demonstrate the formation of bilirubin at the site of the injection of hemoglobin, one may assume from the experiments of van den Bergh, and of Makino,[39] that the hemoglobin underwent such a transformation. It would appear that the cells concerned in this process are the histiocytes, which belong to the same

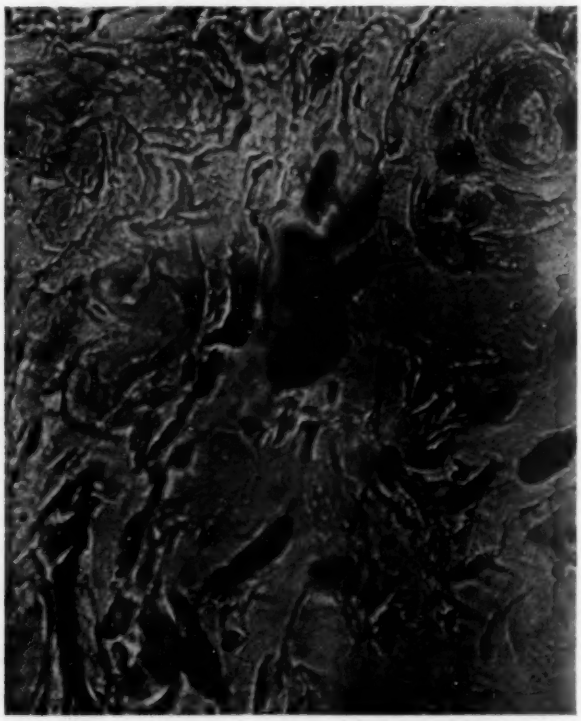

Fig. 8.—Scalp sixteen days after local injection of hemoglobin. Iron is seen in the connective tissue macrophages; × 600.

general system (reticulo-endothelial) as those cells which normally convert hemoglobin into bilirubin or its precursor in the bone-marrow, spleen and liver.

COMMENT

The experiments described in this report are the result of an attempt to use new methods in the study of an old problem, namely, the site of the formation of bile pigment. It was found that such tissues as the

39. Makino, J.: Beiträge zur Frage des anhepatocellulären Gallenfarbstoffbildung, Beitr. z. path. Anat. u. z. allg. Path. **72**:808, 1923-1924.

bone-marrow, spleen and liver contained, in varying amounts, an orange-yellow pigment which gave the characteristic Berlin blue reaction of iron. Since it is known that iron is liberated in the breaking down of hemoglobin into bilirubin, it is reasonable to conclude that the cells that contain iron are those concerned in the conversion of hemoglobin into bilirubin, or at least into a precursor of bilirubin. Furthermore, when samples of the various tissues are removed before the intravenous injection of hemoglobin and at regular intervals of time thereafter, it may be justifiably assumed that the tissues showing a striking increase in iron content (namely, the bone-marrow) are those actively concerned in the process of converting hemoglobin into bilirubin, while those tissues containing a constant amount of iron (as the spleen) probably have to do with storage of the iron until it is again built up into hemoglobin.

Various objections can be raised to the methods used and the conclusions drawn. It is unfortunate that a microscopic demonstration cannot be given of every step in the conversion of the hemoglobin molecule into bilirubin. Instead, one must fill in the gaps with the aid of information obtained by other workers who approached the problem with other methods. The results obtained harmonize well with those reported by experimenters who removed various organs, and by those who studied the blood flowing to and from these organs and tissues. From a review of the various types of cells that contain an increased amount of iron following the injection of hemoglobin (reticular cells of the bone-marrow, stellate cells of the liver and histiocytes in the thoracic wall and scalp), it is seen that these cells are all a part of the reticulo-endothelial system.

CONCLUSIONS

The Berlin blue method for the demonstration of iron-containing pigment in the tissues provides a means of identifying the cells that are concerned with at least one stage of the conversion of hemoglobin into bilirubin.

By the aforementioned method it has been shown that the intravenous injection of hemoglobin is often followed within an hour by a striking increase in the number of droplets of iron-containing pigment in the reticular cells of the bone-marrow, and occasionally by a moderate increase of such pigment in the stellate cells of the liver.

The conclusion is drawn that the bone-marrow plays the major rôle in the formation of bilirubin, with the liver, spleen and possibly the lymph nodes having a lesser part in this process.

The connective tissue macrophages, or histiocytes, are probably responsible for the local formation of bilirubin resulting from the injection of hemoglobin into the pleural cavity or under the scalp.

DOES SPLENECTOMY INFLUENCE THE LEUKOPENIA INDUCED BY THE INJECTION OF CERTAIN FOREIGN SUBSTANCES?*

ISOLDE T. ZECKWER, M.D.
PHILADELPHIA

Doan, Zerfas, Warren and Ames[1] recently reported interesting experiments which suggest that the spleen may play a more inportant rôle in the redistribution of leukocytes within the body after injections of foreign substances than has hitherto been thought to be the case. Sodium nucleinate injected intravenously into a normal animal results in a marked leukopenia in the peripheral blood, which lasts for a number of hours, when it is succeeded by a leukocytosis. Doan and his co-workers expressed the belief that during the peripheral leukopenia the leukocytes have collected exclusively in the spleen, as indicated by leukocyte counts made on blood from the viscera, and they stated: "That the spleen is solely responsible for the temporary depression of white cells in the general circulation under the conditions has been shown by splenectomy." They found that, "with the spleen eliminated from the rabbit, sodium nucleinate, under the conditions outlined, repeatedly produced a leukocytosis, without preceding leukopenia, within a period of time one half to one sixth of that required in animals in which the spleen was intact, the latter invariably showing a profound leukopenia immediately following the nucleinate injection." They indicated the importance of such a function of the spleen: "That the spleen may, under certain conditions, act as a temporary reservoir for myeloid white cells and thus exert something of a regulatory function, more or less beneficial, over their availability to the general circulation and tissues seems clear." They expressed the belief, however, that the "leukopenia of splenic origin after sodium nucleinate" may represent a disadvantage.

Since the peripheral leukopenia following the injection of sodium nucleinate seemed to resemble the peripheral leukopenia commonly observed after the intravenous injection of killed bacteria and other foreign substances, it seemed of importance to determine whether

* Submitted for publication, Feb. 28, 1929.

* From the Department of Pathology, University of Pennsylvania Medical School.

1. Doan, C. A.; Zerfas, L. G.; Warren, S., and Ames, O.: A Study of the Mechanism of Nucleinate-Induced Leucopenic and Leucocytic States, with Special Reference to the Relative Rôles of Liver, Spleen, and Bone Marrow, J. Exper. Med. **47**:403, 1928.

Doan's observations on the effect of splenectomy were of universal application to the reaction to foreign substances in general or whether they applied only to the reaction to the specific substance sodium nucleinate. If the spleen has such a function as storing and then discharging leukocytes, it would be of as great significance as the mobilization of red cells by the spleen from its reservoir, in conditions of great need, as demonstrated by Barcroft.[2]

To determine this point, experiments were carried out, the effect of splenectomy being observed on the peripheral leukocyte count following injections of bacterial vaccines, and the results being compared with those following injections of sodium nucleinate.

EXPERIMENTAL PROCEDURE

Vaccines were prepared by suspending twenty-four hour cultures of *B. coli* on agar in 0.9 per cent sodium chloride and killing by heat. The same suspension was used in 1 cc. doses in all rabbits, into which injections were made. This dosage caused no obvious symptomatic or circulatory changes in the rabbit, and was well tolerated in repeated doses.

Sodium nucleinate (Merck) from yeast nucleic acid, the same product as used by Doan, was dissolved in distilled water in 10 per cent solution. Two lots were used, both being negative to the biuret reaction. In addition, injections were made into one animal of nucleinic acid (Merck) to which sodium hydroxide had been added until solution was effected and was faintly alkaline with phenolphthalein.

Curves of the leukocyte counts of the blood from the ear were made preliminary to and following the intravenous injection of these substances. Splenectomy was carried out on eleven rabbits aseptically. At varying times following operation, the leukocyte response to vaccines was determined in one series of animals, and the response to sodium nucleinate in another series.

To avoid all possible emotional factors which cause a disturbance in the leukocyte count, the rabbits were kept as quiet as possible during the bleeding, on a small table or in a roomy box, which restrained them from running about, but which permitted them to remain in their natural positions without cramping. Leukocyte counts were made by the same person with the same counting apparatus throughout the course of the experiments. Counts were made only after a good flow of blood had been obtained, as experience had shown that counts made on the first drop of blood escaping when the blood flow is poor give figures that are entirely too high. When the ears are cold and cyanotic, as seen after large doses of sodium nucleinate, in the more or less stagnant blood, the leukocytes are likely to accumulate, as is evidenced by the fact that subsequent drops of blood show lower counts, which check each other satisfactorily. This is in accord with the observations of Shaw,[3] who found that when the rabbit's ears are cold, and the blood flow is slow, the leukocytes accumulate in the vessels and cause a higher count than

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2. Barcroft, J.; Harris, H. A.; Orahovats, D., and Weiss, R.: A Contribution to the Physiology of the Spleen, J. Physiol. **60**:443, 1925. Barcroft, J., and Stephens, J. G.: Observations upon the Size of the Spleen, ibid. **64**:1, 1927.

3. Shaw, A. F. B.: The Influence of the Vasomotor State of the Peripheral Blood Vessels on the Leucocytic Content of the Blood, J. Path. & Bact. **29**:389, 1926.

when the blood flows at normal velocity. He also found that pressure or "milking" a constricted vessel caused an increase in the leukocyte count.

After preliminary leukocyte counts were made, the intravenous injection was given, following which leukocyte counts were made at frequent intervals throughout the day. All counts were made on the blood from one ear, while the other ear was used for the intravenous injection. The experiments were all begun at about the same time of day, and the animals were fasted since the day before.

Autopsies on splenectomized animals showed no accessory splenic tissue.

In three splenectomized animals, blood pressure tracings were made with the animals under sodium barbital anesthesia (0.4 Gm. per kilogram of body weight), simultaneous leukocyte curves being made.

RESULTS

Leukocyte Changes Following the Injection of B. coli Vaccine.—(a) The Effect of Splenectomy on the Degree of Leukopenia: The leukocyte curves following the injection of *B. coli* vaccine were compared in two animals (R 1 and R 3, table) before and after splenectomy. A rapid fall in leukocytes to a low level occurred after each injection, whether the spleen was present or absent, even when, as on one occasion, a high initial leukocyte count was present due to infection (R 1). A third rabbit (R 5), in which no curve had been determined before splenectomy, showed on two occasions a profound leukopenia fourteen and nineteen days after splenectomy.

Usually the leukocytes fell to about the same level, whether the bacterial injection was a first injection or a repeated dose, or whether the spleen was present or had been removed. On two occasions, once in an intact rabbit (R 2) and once in a splenectomized rabbit (R 3), however, the leukocytes did not fall to such a low level as they had on a previous injection in the same animal. Consequently, any demonstration that splenectomy causes an alteration in the degree of leukopenia would have to be controlled by determining the range of variation in a large series of intact animals. No such quantitative comparison was attempted in these experiments. For the present purpose, merely the demonstration that a marked leukopenia occurred in the absence of the spleen was considered sufficient.

From the total leukocyte counts and the differential count, the number of granular and nongranular cells was calculated. When the total leukocyte count dropped, the number of polymorphonuclear cells fell nearly to zero, and that of the lymphocytes fell appreciably. When the total leukocyte count rose, this rise was due largely to young forms of polymorphonuclear cells, and the lymphocytes remained at a low level and did not rise during the course of the experiment. The rise in leukocytes was due then to new cells being discharged from the marrow, not to the original leukocytes being returned to the circulation. The same distribution of cells occurred whether the rabbits were splenectomized or intact.

The Effect of the Injection of B. Coli Vaccine and Sodium Nucleinate on the Leukocyte Count in the Peripheral Blood

Animal	Date	Experimental Condition of the Animal	Agent Injected	Leukocyte Count Before Injection	Lowest Count After Injection	Hours After Injection When Leukocytes Began to Rise
R 1	9/13/28	Intact	1 cc. of B. coli vaccine	10,920	2,000	Between 3 and 4
	9/14/28	Splenectomy				
	9/19/28	5 days after operation	1 cc. of B. coli vaccine	16,400	2,700	Between 2 and 2½
	9/26/28	Infection; 12 days after operation	1 cc. of B. coli vaccine	22,800	2,000	Between 2 and 3½
R 2	9/13/28	Intact	1 cc. of B. coli vaccine	9,100	1,000	No rise by 5½
	9/19/28	Intact	1 cc. of B. coli vaccine	8,400	4,400	Between 2 and 3
	9/24/28	Intact	1 cc. of B. coli vaccine	8,400	2,860	
R 3	9/17/28	Intact	1 cc. of B. coli vaccine	10,600	2,060	Between 3½ and 4½
	9/20/28	Splenectomy				
	9/24/28	4 days after operation	1 cc. of B. coli vaccine	12,900	4,200	Between 2¼ and 2¾
R 4	9/17/28	Intact	1 cc. of B. coli vaccine	14,520	3,520	Between 5 and 6½
	9/21/28	Intact	1 cc. of B. coli vaccine	11,800	3,800	
R 5	10/ 5/28	Splenectomy				
	10/19/28	14 days after operation	1 cc. of B. coli vaccine	6,920	1,600	
	10/24/28	19 days after operation	1 cc. of B. coli vaccine	6,640	1,050	
R 6	9/27/28	Splenectomy				
	10/17/28	20 days after operation	1 Gm. of nucleinic acid + sodium hydroxide	10,400	3,200	No rise by 4½
R 7	10/ 5/28	Splenectomy				
	11/22/28	48 days after operation	0.1 Gm. of sodium nucleinate	16,800	2,040	Between 1½ and 2
	12/ 7/28	63 days after operation	1 Gm. of sodium nucleinate	11,240	1,940	Between 4½ and 5
	1/ 2/29	89 days after operation (Sodium barbital anesthesia)	1 Gm. of sodium nucleinate	9,200	2,700	No rise at 1¼
R 8	11/ 5/28	Intact	0.5 Gm. of sodium nucleinate	8,200	3,800	No rise by 1¼
	11/ 8/28	Intact	0.1 Gm. of sodium nucleinate	13,650	2,150	Between 5 and 6
	12/12/28	Intact	0.1 Gm. of sodium nucleinate	7,400	1,200	Between 3¼ and 3¾
	12/21/28	Splenectomy				
	1/ 3/29	13 days after operation	0.1 Gm. of sodium nucleinate	9,000	1,000	Between 4 and 4½
	1/17/29	27 days after operation (Sodium barbital anesthesia)	0.1 Gm. of sodium nucleinate	7,450	1,800	
R 9	11/ 6/28	Splenectomy				
	11/13/28	7 days after operation	0.1 Gm. of sodium nucleinate	8,150	2,950	No rise at 1¼
R 10	11/ 6/28	Splenectomy				
	11/13/28	Infection; 7 days after operation	0.05 Gm. of sodium nucleinate	24,600	9,060	
	11/20/28	Infection; 14 days after operation	0.1 Gm. of sodium nucleinate	27,000	2,600	Between 1 and 2
R 11	11/27/28	Intact	0.5 Gm. of sodium nucleinate	7,060	900	Between 6 and 7
	12/ 5/28	Intact	0.5 Gm. of sodium nucleinate	10,000	1,940	Between 5 and 5½
	12/ 6/28	Splenectomy				
	12/11/28	5 days after operation	0.5 Gm. of sodium nucleinate	14,900	2,300	Between 3 and 3½
R 12	11/26/28	Intact	0.1 Gm. of sodium nucleinate	8,920	1,500	Between 5½ and 6
	12/ 3/28	Intact	0.1 Gm. of sodium nucleinate	8,900	1,400	Between 5¼ and 5½
	12/ 6/28	Splenectomy				
	12/11/28	5 days after operation	0.1 Gm. of sodium nucleinate	7,900	800	Between 6 and 6¼
	1/31/29	28 days after operation (Sodium barbital anesthesia)	0.1 Gm. of sodium nucleinate	8,200	1,200	No rise at 3½
R 13	12/ 4/28	Intact	0.1 Gm. of sodium nucleinate	8,740	1,000	Between 3½ and 4
	12/12/28	Intact	0.1 Gm. of sodium nucleinate	10,000	1,400	Between 5¾ and 6
	12/21/28	Splenectomy				
	1/ 2/29	12 days after operation	0.1 Gm. of sodium nucleinate	11,000	3,400	Between 2½ and 3

(b) The Effect of Splenectomy on the Time of Rise of Leukocytes after the Leukopenic Period, Following the Injection of Vaccine: In R 1 and R 3 (table), the rise in leukocytes after the leukopenic period occurred sooner after splenectomy than in the same animals before splenectomy. Doan and his co-workers had considered that an earlier rise in leukocytes after injection of sodium nucleinate was a result of splenectomy. But in the case of injections of vaccine, in which the development of immunity must be considered, such a conclusion could not be reached without determining the effect of repeated injections per se.

In an intact animal, R 2, two curves following the injections of vaccine were made six days apart. The second injection resulted in a much earlier rise in leukocytes. Consequently, the earlier rise obtained in R 1 and R 3 after splenectomy, as compared with that before operation, could not be attributed to splenectomy, but seemed to be merely the quicker response on the part of the leukocytes induced by the first injection.

The results indicated that splenectomy had no such effect on the reaction to vaccine which was found by Doan and his associates to occur after injection of sodium nucleinate. This conclusion necessitated a reinvestigation of the effect of splenectomy on the leukocyte changes following the injection of sodium nucleinate.

Leukocyte Changes Following the Injection of Sodium Nucleinate.—(a) The Effect of Varying Doses of Sodium Nucleinate: Doan used sodium nucleinate in doses of 1 Gm. per rabbit. In his charts it is indicated that he often used caffeine sodium benzoate with it. Evidently, the animals required stimulants after this large dosage, as, from his description, the general constitutional reaction of the animals to this substance appears to be severe. In the present experiments, doses of 1 Gm. were used in two animals (R 6, R 7) without stimulants, but these animals were so prostrated by the dose that blood could be obtained only with great difficulty. It was found that 0.1 Gm. of sodium nucleinate per rabbit was effective in producing a leukopenia, and resulted in no obvious vascular changes, so that the animals could be bled readily, and this dosage was well tolerated by the animals, so that injections could be repeated frequently for comparison, without stimulants.

(b) The Effect of Splenectomy on the Degree of Leukopenia: In the present experiments, it was found that splenectomy did not prevent the occurrence of a marked degree of leukopenia following the intravenous injection of sodium nucleinate in doses of 1 Gm. per rabbit (R 6, R 7); in doses of 0.5 Gm. (R 11), and in doses of 0.1 Gm. (R 7, R 8, R 9, R 10, R 12, R 13, table). In no animal was there found an absence of a marked degree of leukopenia, and this occurred

at times following splenectomy varying from four to eighty-nine days. Just as with injections of *B. coli*, there could not be made out any clearcut difference in the degree of leukopenia depending on whether it was the first injection or a repeated injection, or whether the spleen was present or had been removed. Even in the presence of a high leukocyte count due to infection in R 10, the leukocyte fall was pronounced. In fact, the higher the leukocyte count, the more spectacular is the fall. With the doses of 1 Gm. it was difficult to get representative samples of blood. The blood as first secured from the puncture was deep purple and viscid. Attention has been called previously to the fact that such blood does not permit a valid count. As concrete illustrations of this, in the splenectomized rabbit R 7, one hour after the injection of sodium nucleinate, a count made on the stagnant blue blood was 4,600 cells, while a moment later, when a good flow had been secured, the count was 2,700.

(c) The Effect of Splenectomy on the Time of the Rise of Leukocytes After the Leukopenic Period: In the experiments of Doan and his associates, they stated that splenectomy results in a rise in leukocytes after injections of sodium nucleinate within a period of time from one half to one sixth of that required in animals with spleens intact. Perhaps the results in one group of splenectomized rabbits were being compared with those in a control group of intact animals rather than the curves in the same animals before and after splenectomy, as the charts of the splenectomized animals show no curves previous to the operations, except that in one chart the curve after partial splenectomy was compared with that after complete splenectomy in the same rabbit.

In the present experiments, the time of rise in leukocytes was found to differ so greatly in different intact animals that it would seem to be necessary to have a large series of normal and test animals, in order to establish means of the two series which could be compared for a significant difference. Instead of running such a large series, it was thought advisable to determine at least two curves before splenectomy in several animals, and to compare these results with curves after splenectomy in the same animals. This would control the effect of repetition of the injection and would also control the factor of individual variation in different animals. The accompanying table shows the results thus obtained in R 8, R 11, R 12 and R 13 (charts 1 and 2).

R 11 received two injections before splenectomy was performed. The second time a rise occurred nearly two hours sooner than on the first injection. The animal was then splenectomized. Five days after operation, on the injection of the same dose of sodium nucleinate, the rise occurred nearly two hours sooner than on the second injection. That

is, the interval had not been shortened after splenectomy by any more than that by which the second response, when the spleen was intact, had been shortened over the first. R 12, on the other hand, showed almost the identical interval on two occasions before splenectomy and at five days after splenectomy.

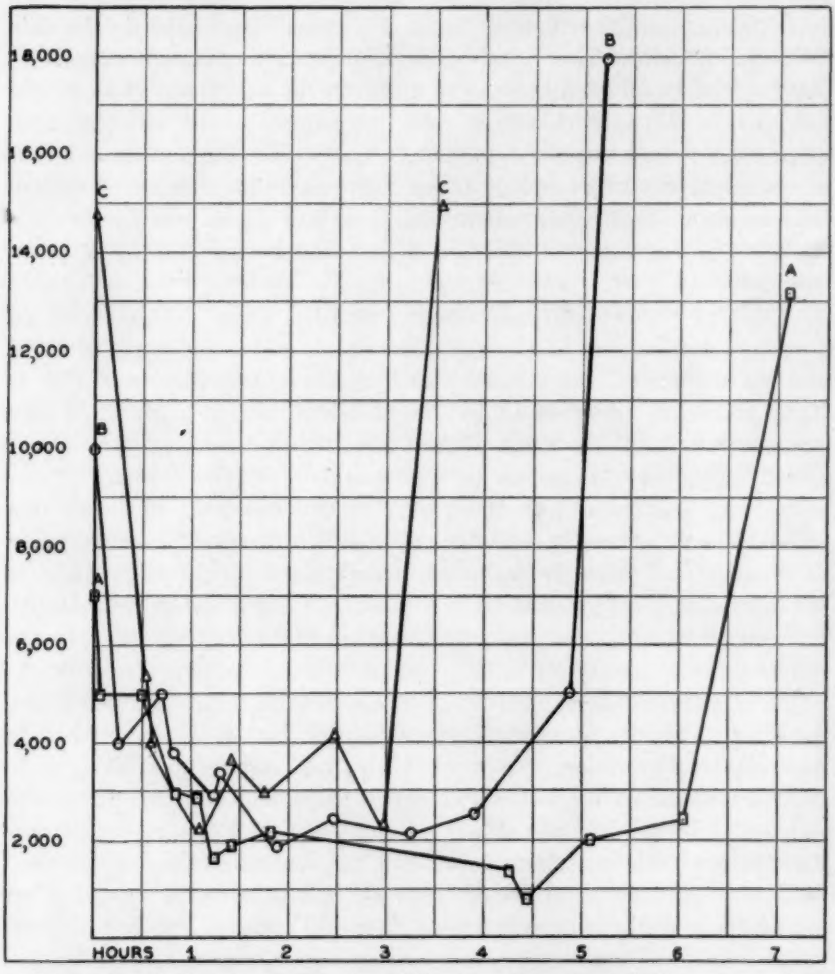

Chart 1.—Leukocyte counts in rabbit 11 after the injection of sodium nucleinate. The curve marked *A* represents the leukocyte count on November 27, after the injection of 0.5 Gm., with the animal intact; *B* represents the count on December 5, after the injection of 0.5 Gm., with the animal intact, and *C*, that on December 11, after an injection of 0.5 Gm., five days after splenectomy.

R 8 showed a return rise in leukocytes about two hours sooner after the third injection before splenectomy than after the second. After splenectomy, the rise occurred later than on the third occasion before splenectomy.

R 13 was the only rabbit that showed an earlier rise after splenectomy than at any time before splenectomy. In the present experiments, therefore, there was no clearcut evidence that splenectomy resulted in a more rapid return rise of leukocytes.

(d) The Changes in Blood Pressure Resulting from the Injection of Sodium Nucleinate: The question arose as to whether the difference

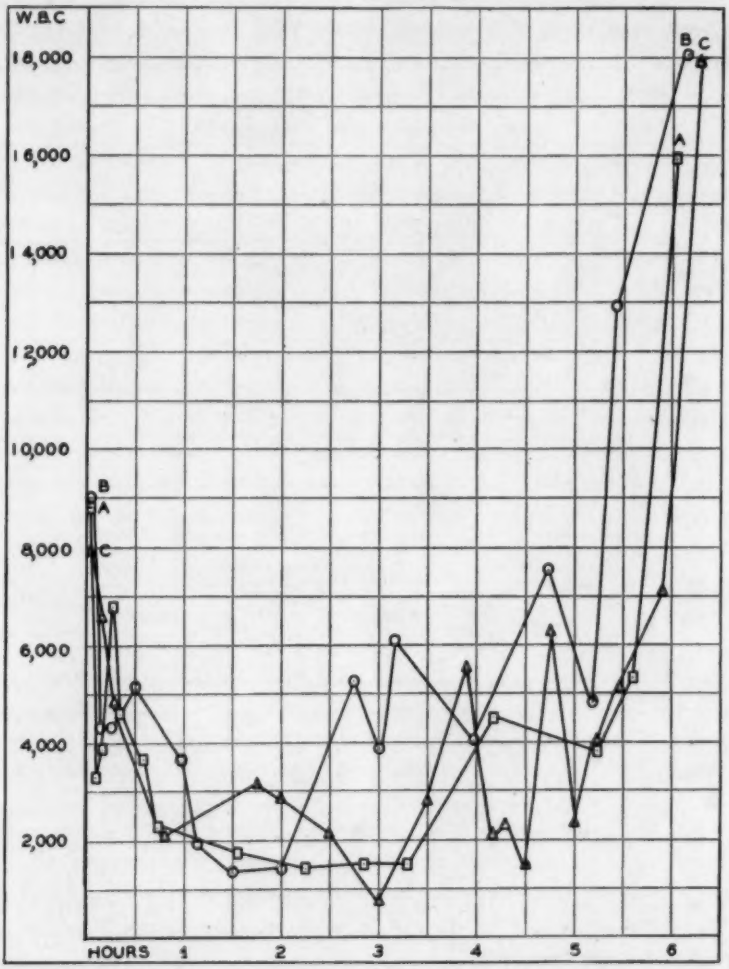

Chart 2.—Leukocyte counts in rabbit 12 after the injection of sodium nucleinate. The curve marked *A* represents the count on November 26, after the injection of 0.1 Gm., with the animal intact; *B*, that on December 6, after the injection of 0.1 Gm., with the animal intact, and *C*, that on December 11, after the injection of 0.1 Gm., five days after splenectomy.

in results obtained by Doan and his associates and those recorded in the present experiments was dependent on differing conditions, such as changes in blood pressure occurring in the present experiments and not

operating in Doan's experiments. Doan reported that the blood pressure was recorded in one cat after an injection of 2 Gm. of sodium nucleinate; a moderate rise was maintained for some time. As no leukocyte counts are stated, it is not evident that a leukopenia occurred in this experiment with the dosage used. Vasoconstriction and vasodilation occurred in the rabbits, and, as previously stated, these investigators often used caffeine sodium benzoate, which implies that the animals may have been in a state of considerable shock.

Records of the blood pressure were made in splenectomized rabbits (R 7, R 8, R 12), with the animals under sodium barbital anesthesia

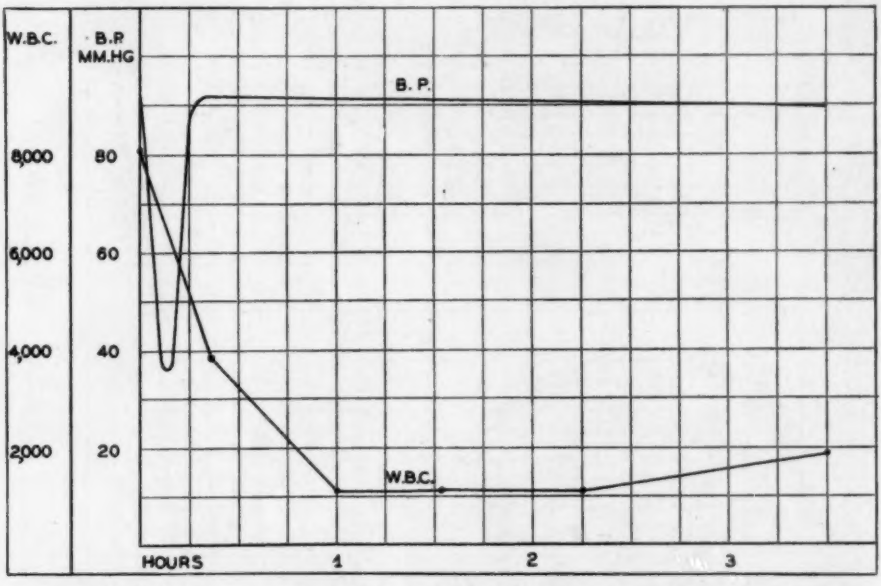

Chart 3.—Leukocyte counts (W. B. C.) and blood pressure (B. P.) following the injection of 0.1 Gm. of sodium nucleinate into rabbit 12 (splenectomized).

(0.4 Gm. per kilogram of body weight). Every precaution was taken to guard against complicating factors, so that the changes in blood pressure that occurred were the result of injections of sodium nucleinate per se. Leukocyte curves of the blood from the vein of an ear were made at the same time.

In R 7, the injection of 1 Gm. of sodium nucleinate caused an abrupt fall in blood pressure from 80 to 30 mm. of mercury. The blood pressure continued at the same low level without any rise for one and a half hours, when the experiment was terminated. The number of peripheral leukocytes fell to 2,700, but this probably does not represent the lowest level, as counts were not made frequently.

In R 12, the smaller dosage, 0.1 Gm. of sodium nucleinate, resulted in a temporary fall of blood pressure from 92 to 36 mm. of mercury, but there was a rapid return to the previous level in nineteen minutes. For three and a half hours thereafter the blood pressure remained at the level at which it was previous to injection; the experiment was then terminated. The leukopenia occurred after the blood pressure had returned to normal, and was maintained, the counts varying from 1,200 to 1,800 cells during this entire period. Chart 3 represents the changes in blood pressure.

A similar result was obtained with the smaller dose in R 8, in which the blood pressure before injection was 110 mm. On the injection of 0.1 Gm. of sodium nucleinate, the blood pressure fell temporarily to 40 mm., with a rapid return to the previous level in eleven minutes. The leukopenia was maintained for two and a half hours, during which time the blood pressure remained at the preliminary high level until the experiment was terminated.

In the present experiments, five rabbits were used for injections of *B. coli* and eight for injections of sodium nucleinate. Three of the animals in the *B. coli* group and eight in the sodium nucleinate group were splenectomized. The uniformity of the results obtained made further experiments seem unnecessary. In Doan's experiments, "uncomplicated studies of the peripheral blood after sodium nucleinate were made in six rabbits," and splenectomy was performed in seven rabbits.

COMMENT

The literature on the problem of the redistribution of leukocytes within the body after the injection of various agents has been discussed fairly completely by Doan and his co-workers; so it need not be mentioned here. These authors emphasized the necessity of studying the living animal with the circulatory system functioning normally, and they believed that disregard of this is responsible for the conflicting data in previous experiments, many of which have been histologic studies of the tissues at death. In living rabbits under barbital anesthesia they found, during the period of peripheral leukopenia, lowered leukocyte counts in the blood from the liver and lungs and no evidence of the accumulation of leukocytes within these organs, but a marked accumulation in the spleen.

In the present experiments, no attempt was made to localize accurately the leukocytes during the period of peripheral leukopenia. That peripheral leukopenia was invariably demonstrated in splenectomized animals implies logically that the viscera or the vascular system in the splanchnic area provide for the temporary storage of leukocytes, and that such function completely compensates for whatever function the spleen possesses in this regard.

The conflicting results of these experiments with those of Doan illustrate the complexity of factors which determine the leukocyte count in the peripheral blood at any moment. There is no suggestion that the actual quantitative data are not correct, in both instances, under the given experimental conditions. But how slight may be the difference in experimental conditions which may be responsible for varying results is clearly demonstrated. I have not been able to find any other work published on the effect of splenectomy on the redistribution of leukocytes which throws light on the factors that might explain the apparent discrepancy. I am inclined to believe that the differences in results may depend on the vasomotor state of the peripheral vessels, the use of caffeine perhaps determining the difference in Doan's experiments.

SUMMARY

Under the conditions of these experiments, splenectomy in rabbits had no effect in preventing the occurrence of the usual leukopenia which results from the intravenous injection of *B. coli* vaccine and of sodium nucleinate, and had no definite effect in hastening the return rise in the number of leukocytes after the leukopenic period.

SOLITARY CYST OF THE KIDNEY*

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Solitary cysts of the kidney are one of the most uncommon conditions encountered in renal pathology. In a recent paper, Carson [1] collected reports of 151 cases from the literature. But Kairis,[2] who also reviewed the literature on this subject, stated that Laquière,[3] to whom Carson referred in his communication, erroneously included in his list of solitary cysts a number of cases which do not belong to this group, but which consist of echinococcus cysts, paranephritic cysts, cystic kidneys, traumatic blood cysts and other growths.

SEX AND AGE

Solitary cysts are more often found in women than in men (Carson gave a ratio of 89:41). The same author notes further that they usually occur in persons from 30 to 60 years old (average age, 45). But they are occasionally seen in young persons and in old people.

LOCATION AND MICROSCOPIC APPEARANCE

The cysts are in general unilateral and are more often located in the right kidney than in the left (Carson gave a ratio of 51:21). They may occupy the upper or the lower pole (Kairis found the cysts in the lower pole in two thirds of the cases) and are infrequently found in the anterior surface or the hilum.

They appear in the hypochondriac region as round or irregularly oval cystic formations attached with a more or less broad base to the kidney. They project into the abdominal cavity, where they are sometimes adherent to the adjacent organs. The surface is grayish white and smooth. The wall may be translucent. They vary in size from that of a hen's egg to that of a full term uterus (from 30 to 6,000 cc.). Their average diameter is 15 cm. (Carson). The thickness of the wall varies from 0.5 to 3 cm. The fibrous capsule of the kidney extends over the whole cyst and can often be separated from the cystic wall proper. The inner surface is smooth, grayish white and glistening.

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* Submitted for publication, March 7, 1929.

* From the Department of Pathology, Loyola University School of Medicine and the Laboratories of Mercy Hospital.

1. Carson: Solitary Cysts of the Kidney, Ann. Surg. **87**:250, 1928.

2. Kairis: Zur Kenntnis der solitären Nierencysten, Arch. f. klin. Chir. **149**: 700, 1928.

3. Laquière: Kystes séreux du rein et opérations conservatrices, J. de chir. **26**:257, 1925.

Trabeculation is sometimes observed covering the whole or parts of the inner surface. Multilocular cysts are occasionally seen. The contents usually consist of a clear, yellow, thin fluid which is strongly albuminous and contains urea, uric acid, chlorides and infrequently also cholesterol and fat. Contents of a bloody character are due to a secondary hemorrhage (Laquière found this in one tenth of the cases). Secondary infection may result in a purulent fluid. Besides crystals, the sediment may contain desquamated epithelial cells from the lining of the cyst. Communications of the lumen of the cyst with the renal pelvis or the calices do not exist.

MICROSCOPIC APPEARANCE

The wall in general consists of three layers. The outer layer is formed by either the fibrous renal capsule or by atrophic renal tissue; the middle layer is a loose, vascular connective tissue, and the inner layer consists of cells ranging in shape from flat ones to cuboidal epithelial ones. In the majority of the cases, the epithelial lining was absent. The middle layer does not contain any elastic tissue except that found in the vascular walls. In a recent publication, Judd and Simon [4] stressed this fact; they contended that solitary cysts with hemorrhagic contents are not always solitary serous cysts with secondary hemorrhage into the lumen, but that they are partly primary formations originating from aneurysms, as they contain elastic tissue in their wall.

ETIOLOGY

There are several theories concerning the etiology of these cysts. The retention theory supported by Virchow [2] contends that the solitary cysts result from a constriction of tubules by scar tissue or blocking of the lumina by desquamated and degenerated tubular epithelium and contributory small hemorrhages. The absence of chronic inflammatory changes in many of the cases reported does not favor this theory. In another theory, a failure of the union between glomeruli and tubuli during the embryonal development is considered as the causative mechanism of the cysts. Borst,[2] on the other hand, regarded the cysts as the result of independent new formations which arise from embryonal rests. In support of this theory, which is not accepted by Lubarsch,[2] Ruckert [2] reported the existence of transitions between solitary cysts and polycystic kidneys. Sonntag [2] expressed the belief that they may originate from primary solid tumors due to extensive central necrosis. A new view is brought into the discussion by the investigations of Kampmeier.[5] In the study of fetal kidneys, he observed that during

4. Judd and Simon: Hemorrhagic Cysts of the Kidney, Surg. Gynec. Obst. **45**:601, 1927.

5. Kampmeier: A Hitherto Unrecognized Mode of Origin of Congenital Renal Cyst, Surg. Gynec. Obst. **36**:208, 1923.

this period the tubules normally pass through a cystic stage. He contended, therefore, that the persistence of this state during later life represents the cause of solitary cysts.

REPORT OF CASE

A woman, aged 53, came to autopsy after an acute illness of a few days. She had never complained of any abdominal symptoms. The autopsy showed a stout woman with a diffuse, bilateral, hemorrhagic bronchopneumonia, fatty degeneration of the heart, septic hyperplasia of the spleen, cloudy swelling of the liver and kidney, marked hyperemia of the meninges and the brain, two small polypi in the uterine cervix and a large solitary cyst of the left kidney and a small one of the right.

The solitary serous cyst of the left kidney was located in the upper pole and measured 17 by 12 by 7 cm. It was oval, with a delicate, translucent, grayish-white capsule in which blood vessels radiating from the kidney were clearly visible. The renal capsule covered the cyst and could be stripped easily. The base by which the cyst was attached to the kidney measured about 7 by 4 cm., and the kidney itself, 12 by 7 by 6 cm. The kidney showed two deep retractions on the surface and was somewhat curved toward the anterior abdominal wall. The inside of the cyst was smooth and glistening and showed several low trabeculae, which crossed each other in irregular fashion. There was no connection with the renal pelvis. The cyst contained a clear, serous, somewhat yellowish fluid. The chemical analysis showed: albumin; sugar, 20 mg. per hundred cubic centimeters; non-protein nitrogen, 25.8 mg.; uric acid, 2.3 mg.; urea nitrogen, 7.4 mg.; creatinine, 1.1 mg., and no cholesterol. The sediment showed only amorphous crystals.

The small cyst the size of a hazelnut in the cortex of the right kidney was located at the middle of the outer curvature and contained a clear, watery fluid.

The microscopic examination of the wall revealed atrophic kidney tissue on the outside and a vascular, loose connective tissue on the inside of the cyst. An epithelial lining was absent. There was no elastic tissue in the cystic wall. Examination of the kidney at the region of the deep retraction showed a disturbance in the regular arrangement of the tubuli and glomeruli. There was no increase of the interstitial fibrous tissue nor any evidence of a chronic inflammatory process in these areas.

SUMMARY

A case of solitary serous cyst of the kidney, which remained clinically latent during life, is reported. The presence of structural irregularities in the kidney seems to indicate that developmental disturbances are responsible for the origin of solitary serous cysts.

CHANGES IN THE LIVER AND IN THE PANCREAS IN CHRONIC PULMONARY TUBERCULOSIS

WITH SPECIAL REFERENCE TO THE ISLETS OF LANGERHANS *

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It is well known that certain morphologic changes occur in the liver in cases of chronic tuberculosis of the lung. Aside from the frequent finding of tubercles, infiltration by fat and an increase in connective tissue in the liver often seem to accompany tuberculosis of the lung. The pancreas in these cases rarely shows tubercles, and only infrequently an increase in connective tissue. The correlation between the changes in the pancreas and those in the liver in cases of chronic tuberculosis of the lung, however, has received but little attention.

The study here presented deals with the examination of the liver and the pancreas in 100 cases of chronic ulcerative tuberculosis of the lung and with the data taken from 767 cases of tuberculosis. Attention was directed to infiltration of the liver by fat, the interstitial tissue of the liver and pancreas and especially to the islets of Langerhans. Other changes in the liver and pancreas, as parenchymatous changes, passive hyperemia and amyloidosis, were noted but are not discussed.

MATERIAL AND METHODS

One hundred cases of chronic ulcerative tuberculosis were used for this study. The patients were inmates of the sanitarium which is part of the Cleveland City Hospital. The autopsies were performed from three to twenty-four hours after death. Blocks of the liver and of the tail, the head and the midportion of the pancreas were hardened in a diluted solution of formaldehyde, U. S. P. (1:10) and imbedded in paraffin. Several blocks were used for frozen sections, and stained for fat with sudan III. Hematoxylin and eosin were used for routine stains. Mallory's and van Gieson's stains were applied to demonstrate connective tissue. Ziehl-Neelsen's method was applied to disclose tubercle bacilli. An attempt to count the number of islets of Langerhans was futile; therefore the diameters of the islets were measured with the aid of a filar ocular, and the longest diameters recorded.

LIVER

Infiltration by Fat.—The textbooks of pathology mentioned two conditions as causes of infiltration of the liver by fat, namely, chronic alcoholism and tuberculosis. Indeed, the liver in chronic tuberculosis serves as the every day example of infiltration of that organ by fat.

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Kern and Gold,[1] Lorentz [2] and more recently Spring [3] reported the frequent finding of fat in the liver in cases of tuberculosis. Of ninety-one cases mentioned in Spring's paper, fourteen showed complete infiltration of the liver by fat. In sixteen cases, the infiltration by fat was almost complete, and in thirty-one cases fat was found in an area corresponding to about one third of the lobule. Fifteen cases showed fat only in the periphery of the lobule, while another fifteen cases showed only traces of fat.

Table 1 gives the sex, age, color and external appearance of the patients. It shows in how many cases the liver was the seat of a passive hyperemia, of an increase in connective tissue, of infiltration by fat and of tubercles. It states whether or not ulcerative lesions were present in the intestines, and mentions the conditions in the lungs. It gives, further, the largest diameter of the islets of Langerhans in the pancreas.

Three of the 100 cases showed complete infiltration of the liver by fat. In four, the liver showed fat only in an area extending about halfway from the periportal spaces toward the central vein; in twelve, fat was found in the outer third of the lobules, and in fifteen, fat was present only in the liver cells surrounding the periportal spaces. Thirty-four per cent of the cases had some fat in the liver; only 3 per cent showed complete infiltration by fat. Sixty-six cases presented no fat. In addition to the 100 cases which were examined in detail with the special purposes of this study in mind, the available microscopic sections of the liver and the records of the autopsies in 767 other cases of chronic tuberculosis were examined for infiltration of the liver by fat. In 129 of these 767 cases fat was seen grossly and microscopically, while in twenty-three fat could be detected only microscopically; in other words, only 26.32 per cent of 767 cases showed the presence of fat. No attempt was made to determine the degree of infiltration by fat in this series of cases.

As table 1 further indicates, the age of the patient, the sex and the color apparently have no influence on the presence or absence of fat in the liver. There is no relationship between the external appearance of the body and the fat content of the liver.

In twenty-seven cases, a more or less severe passive hyperemia was present in the liver microscopically. None of these cases showed fat in the liver.

It is surprising that the infiltration of the liver by fat in this material was much less frequent than is described in the literature. The Amer-

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1. Kern, W., and Gold, E.: Ueber die Beziehung von Leberzirrhose zur Tuberkulose, Virchows Arch. f. path. Anat. **222**:78, 1916.

2. Lorentz, F. H.: Die Leber in ihrem Verhalten zur Tuberkulose und Cirrhose, Ztschr. f. Tuberk. **20**:232, 1913.

3. Spring, K.: Die Leber bei Tuberkulose, Frankfurt. Ztschr. f. Path. **32**: 32, 1925.

TABLE 1.—*Summary of Observations in One Hundred Cases of Tuberculosis of the Lungs*

Case	Sex	Age	Color	State of Nourishment	Liver			Tubercles	Tuberculous Ulcers of Intestines	Largest Diameter of Islets of Pancreas, Microns
					Passive Hyperemia	Connective Tissue	Portion Infiltrated by Fat			
1	F	37	B	Fair	Absent	Increase	⅓ *	Absent	Absent	300
2	F	27	W	Good	Absent	Increase	⅓	Present	Present	300
3	M	54	W	Fair	Present	Increase	Absent	Present	Absent	330
4	F	22	W	Good	Absent	Increase	Absent	Present	Present	300
5	M	39	W	Good	Present	Absent	Absent	Absent	Absent	380
6	M	58	W	Good	Present	Sclerosis	Absent	Present	Present	210
7	M	41	W	Good	Absent	Increase	Periportal spaces	Present	Absent	235
8	M	49	W	Good	Present	Absent	Absent	Present	Present	384
9	M	25	W	Good	Absent	Absent	Periportal spaces	Present	Present	228
10	M	39	W	Good	Absent	Increase	Periportal spaces	Present	Absent	265
11	M	38	W	Good	Absent	Increase	Periportal spaces	Present	Absent	296
12	M	31	B	Good	Absent	Increase	Absent	Absent	Absent	300
13	F	25	W	Good	Absent	Absent	Complete	Absent	Present	275
14	M	35	W	Good	Absent	Absent	Absent	Absent	Present	300
15	F	27	W	Good	Absent	Absent	Absent	Present	Absent	225
16	M	38	B	Good	Absent	Absent	Absent	Present	Present	360
17	M	50	W	Good	Absent	Increase	Periportal spaces	Present	Absent	270
18	F	32	B	Poor	Absent	Absent	Absent	Present	Present	305
19	M	20	B	Poor	Present	Increase	Absent	Present	Present	300
20	M	34	W	Fair	Present	Increase	Absent	Present	Absent	300
21	M	27	W	Fair	Absent	Absent	Complete	Present	Present	210
22	F	41	W	Good	Absent	Increase	⅓	Present	Present	180
23	F	29	B	Fair	Absent	Increase	⅓	Absent	Present	300
24	M	36	W	Good	Absent	Increase	Absent	Present	Present	435
25	M	54	W	Good	Present	Increase	Absent	Present	Absent	390
26	F	33	B	Good	Absent	Increase	Absent	Present	Present	435
27	M	20	B	Good	Present	Increase	Absent	Absent	Present	180
28	F	22	B	Good	Absent	Absent	½ †	Present	Present	165
29	M	23	B	Good	Absent	Absent	Absent	Absent	Absent	485
30	F	39	W	Good	Absent	Increase	Periportal spaces	Present	Present	195
31	M	48	B	Good	Absent	Increase	Absent	Present	Present	415
32	F	30	B	Good	Absent	Increase	⅓	Present	Present	345
33	M	46	W	Good	Absent	Increase	⅓	Present	Absent	225
34	F	25	B	Fair	Absent	Increase	⅓	Absent	Present	300
35	M	34	B	Good	Present	Absent	Absent	Present	Absent	265
36	M	60	W	Poor	Present	Absent	Absent	Present	Absent	270
37	F	20	W	Fair	Absent	Absent	Absent	Present	Present	270
38	M	34	W	Good	Absent	Increase	Periportal spaces	Absent	Absent	300
39	F	65	W	Poor	Absent	Sclerosis	Absent	Present	Absent	270
40	F	32	B	Poor	Absent	Absent	Absent	Present	Present	300
41	M	40	B	Good	Present	Increase	Absent	Present	Absent	270
42	M	21	B	Fair	Absent	Increase	Complete	Present	Absent	270
43	M	74	W	Fair	Absent	Absent	⅓	Present	Absent	300
44	M	24	B	Good	Present	Absent	Absent	Present	Absent	275
45	F	60	B	Good	Absent	Increase	Periportal spaces	Present	Present	315
46	M	21	W	Poor	Absent	Increase	Absent	Present	Present	210
47	M	40	B	Fair	Absent	Absent	Periportal spaces	Present	Present	300
48	F	35	B	Poor	Absent	Absent	Periportal spaces	Present	Present	300
49	M	57	W	Good	Absent	Absent	Absent	Absent	Absent	230
50	F	16	B	Fair	Absent	Increase	Absent	Present	Present	330
51	F	30	W	Poor	Absent	Increase	½	Present	Present	165
52	M	36	W	Fair	Present	Increase	Absent	Present	Present	295
53	M	25	B	Good	Absent	Absent	Periportal spaces	Present	Present	270
54	F	29	W	Fair	Absent	Increase	½	Present	Present	270
55	M	54	W	Poor	Absent	Increase	Absent	Present	Absent	300
56	M	40	W	Good	Present	Increase	Absent	Present	Absent	300
57	M	15	W	Good	Present	Increase	Absent	Present	Present	315
58	M	47	W	Fair	Absent	Increase	⅓	Present	Present	180
59	M	24	W	Good	Absent	Increase	Absent	Present	Absent	315
60	M	32	W	Fair	Absent	Increase	Absent	Absent	Present	300
61	F	20	B	Poor	Absent	Increase	Absent	Present	Absent	450
62	M	46	B	Good	Present	Increase	Absent	Present	Present	285
63	M	33	B	Poor	Present	Increase	Absent	Present	Present	285
64	F	17	B	Fair	Absent	Absent	½	Present	Present	300

* ⅓ of the liver lobule.
† ½ of the liver lobule.

TABLE 1.—*Summary of Observations in One Hundred Cases of Tuberculosis of the Lungs—Continued*

Case	Sex	Age	Color	State of Nourishment	Liver				Tuberculous Ulcers of Intestines	Largest Diameter of Islets of Pancreas, Microns
					Passive Hyperemia	Connective Tissue	Portion Infiltrated by Fat	Tubercles		
65	F	20	B	Poor	Absent	Absent	⅛	Present	Present	185
66	M	32	B	Poor	Absent	Absent	Absent	Present	Absent	330
67	M	30	B	Poor	Absent	Increase	Absent	Present	Present	285
68	F	31	B	Good	Absent	Absent	Absent	Absent	Present	495
69	F	36	W	Good	Present	Increase	Absent	Present	Present	225
70	M	50	W	Good	Present	Increase	Absent	Present	Present	330
71	F	27	W	Poor	Absent	Increase	Absent	Present	Present	520
72	M	75	W	Good	Absent	Increase	Absent	Present	Absent	365
73	M	22	W	Good	Present	Increase	Absent	Present	Present	380
74	M	48	B	Good	Absent	Increase	Absent	Absent	Absent	285
75	M	37	W	Poor	Absent	Increase	Absent	Absent	Present	385
76	M	29	W	Good	Absent	Increase	⅛	Present	Present	300
77	F	45	W	Fair	Absent	Increase	Absent	Present	Present	375
78	M	56	W	Good	Absent	Absent	Absent	Present	Present	225
79	M	36	B	Good	Present	Increase	Absent	Present	Present	315
80	M	43	W	Good	Present	Increase	Absent	Present	Present	375
81	M	43	B	Good	Present	Increase	Absent	Present	Present	345
82	M	50	B	Good	Absent	Sclerosis	Absent	Absent	Absent	400
83	M	35	W	Good	Absent	Increase	Absent	Present	Present	300
84	F	32	W	Good	Absent	Absent	Periportal spaces	Present	Present	285
85	M	46	W	Good	Absent	Absent	Absent	Present	Present	330
86	M	40	W	Good	Absent	Increase	Absent	Present	Present	307
87	M	53	W	Good	Absent	Increase	Absent	Present	Present	388
88	M	35	W	Good	Present	Increase	Absent	Present	Present	386
89	M	43	B	Good	Present	Increase	Absent	Present	Absent	315
90	M	37	B	Poor	Absent	Increase	Absent	Present	Present	300
91	F	31	B	Good	Absent	Increase	Absent	Present	Present	511
92	F	32	W	Poor	Absent	Increase	Absent	Present	Present	300
93	F	20	B	Good	Present	Increase	Absent	Present	Present	315
94	M	20	B	Good	Absent	Absent	Absent	Present	Present	367
95	F	24	W	Fair	Absent	Absent	⅛	Absent	Absent	215
96	M	32	W	Good	Present	Increase	Absent	Absent	Present	450
97	F	18	W	Fair	Absent	Absent	Periportal spaces	Present	Present	270
98	F	39	B	Good	Absent	Absent	Periportal spaces	Present	Present	300
99	M	40	W	Good	Absent	Absent	Periportal spaces	Absent	Absent	300
100	M	37	W	Good	Absent	Sclerosis	Absent	Absent	Present	315

ican literature contains few references to this subject, most of the investigations having been made in Europe. Lavenson and Karsner,[4] whose material was taken largely from the Philadelphia General Hospital, described the liver of tuberculous persons with special reference to periportal fibrosis. These authors gave a short description of their general observations of these livers. They mentioned only six of fifty as showing the presence of fat, and in only two of these was the infiltration by fat complete. These observations correspond with my results. The climate and diet may have some bearing on the presence or absence of fat in the liver, and this may account, in part, at least, for the difference in the observations of European and American investigators.

It may be mentioned in this connection that Begtrup[5] (Denmark) stated that previous to 1916, an infiltration of the liver by fat was found in from 18 to 23.5 per cent of all cases in which autopsies were performed;

4. Lavenson, R. S., and Karsner, H. T.: Periportal Fibrosis of the Liver in Tuberculosis, Univ. Penn. M. Bull. **22**:167, 1909-1910.

5. Begtrup, E.: Fat Infiltration of Liver, Ugesk. f. Laeger **82**:1199, 1920; abstr., J. A. M. A. **75**:1686 (Dec. 11) 1920.

but after this time in only from 4.5 to 6.2 per cent. This suggests that the reduced food value during the later years of the World War influenced to a certain degree, at least, the fat content of the liver. In cases of tuberculosis, the type of foods, too, therefore, should be taken into consideration in discussing the cause of infiltration of the liver by fat.

The cause of infiltration of the liver by fat is not known. Various theories are offered to explain the presence of fat in the liver, but none is convincing. Probably the latest communication on this subject is an article by Clauberg,[6] who believed that a lipolytic insufficiency in the liver is a necessary factor in causing the infiltration by fat. After referring to the changes in the pancreas, I shall return to this subject.

Tubercles of the Liver.—Tubercles were found in the liver in eighty of the 100 cases. Since it is practically impossible to examine the entire liver histologically, it may be stated that in at least 80 per cent of the cases examined, tubercles were found in the liver. Lorentz [2] found tubercles in the liver in 99 per cent of his cases, Simmonds [7] in 82 per cent and Torry [8] in 63 per cent, while Lavenson and Karsner [4] found them in only 36 per cent. Fifty-seven of the eighty cases of tubercles of the liver in my series showed ulcerative lesions of the intestines,[9] while in ten cases of intestinal tuberculosis no tubercles were seen in the liver. This observation, however, as mentioned before, must be taken cautiously, because of the possibility of overlooking tubercles of the liver. In twenty-seven cases, tubercles were seen in the liver with infiltration by fat. Two of the three livers showing complete infiltration by fat were the seat of tubercles. The tubercles in the livers that showed fat seemed to be more abundant, to attain a greater size and to show a greater tendency toward fusion by confluence, than tubercles in livers without fat. Since the tubercles were not counted and no measurements of their size were made, this observation should be confirmed.

Connective Tissue.—Increase in connective tissue in the liver, periportal fibrosis and even cirrhosis have often been described as associated with ulcerative tuberculosis. Lavenson and Karsner,[4] Kirch [10] and Spring [5] reviewed the literature in detail. While most of the authors

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6. Clauberg, K. W.: Weitere Mitteilung zum Problem der Fettleber bei Lungenschwindsucht, Virchows Arch. f. path. Anat. **262**:74, 1926.

7. Simmonds, M.: Beiträge zur Statistik und Anatomie der Tuberkulose, Deutsches Arch. f. klin. Med. **27**:448, 1880.

8. Torry, R. G.: The Occurrence of Miliary Tuberculosis of the Liver in the Course of Pulmonary Tuberculosis, Am. J. M. Sc. **151**:549, 1916.

9. This diagnosis was made grossly.

10. Kirch, E.: Ueber tuberkuloese Leberzirrhose, tuberkuloese Schrumpfniere und analoge Folgeerscheinungen granulierender tuberkuloeser Entzuendungen in Pankreas und Mundspeicheldruesen, Virchows Arch. f. path. Anat. **225**:129, 1918.

agreed that there is, as a manifestation of tuberculosis, an increase of varying degree in the fibrous elements of Glisson's capsule, they still disagreed as to whether cirrhosis of the liver is on a tuberculous basis. While MacCallum [11] classified tuberculous cirrhosis as a special group, Epplen [12] did not mention tuberculosis as an etiologic factor in cirrhosis of the liver. Lavenson and Karsner [4] emphasized that tuberculosis is one of the factors leading to a proliferation of connective tissue, and that these observations harmonize with the view held by some observers that tuberculosis is a frequent cause of cirrhosis of the liver. Two of their fifty cases showed cirrhosis of the liver. Schoenberg [13] held that tuberculous cirrhosis of the liver is a frequent observation, while Kirch,[10] and Kern and Gold [1] thought it rare. Kaufmann [14] was of the opinion that the changes in the liver due to tuberculosis may, in some cases, be similar to those found in Laennec's cirrhosis. Lorentz [2] found among 100 cases of cirrhosis, sixteen which should be interpreted as tuberculous in origin. Of 120 patients with cirrhosis of the liver studied by Blumenau,[15] 10.31 per cent died of tuberculosis. Merklen [16] and his co-workers found many tubercles in their case of cirrhosis of the liver. Spring,[3] however, did not believe that a true Laennec's cirrhosis is ever caused by tuberculosis. Huebschmann [17] recently stated that a combination of cirrhosis of the liver and tuberculosis is rare.

Sixty-seven cases of my series showed a proliferation of connective tissue. In only twenty-two cases was the increase confined to the periportal spaces. Forty-three showed an extension into the interlobular space, four of which showed a complete encircling of the lobule. The newly formed fibers of connective tissue were rich in nuclei. All the cases of fibrosis showed an infiltration mainly by lymphocytes, with a few polymorphonuclear leukocytes and endothelial cells. In the four more advanced cases there was a slight proliferation of young bile ducts, partly with and partly without lumina, and a new formation of blood capillaries. These cases also showed a slight regeneration of liver cells, and even a new formation of liver lobules. In short, the liver in these

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11. MacCallum, W. G.: A Textbook of Pathology, Philadelphia, W. B. Saunders Company, 1924.

12. Epplen, F.: The Pathology of Cirrhosis of the Liver, Arch. Int. Med. **29**:482 (April) 1922.

13. Schoenberg, S.: Lebercirrhose und Tuberkulose, Beitr. z. path. Anat. u. allg. Path. **59**:601, 1914.

14. Kaufmann, E.: Lehrbuch der speziellen pathologischen Anatomie, Berlin, 1922.

15. Blumenau, E.: Ueber Todesursache bei Lebercirrhose, Arch. f. Verdauungskr. **18**:1, 1921.

16. Merklen, P.; Turpin, and Dubois-Roquebert: Un cas de cirrhose tuberculeuse hypertrophique, Bull. et mém. Soc. méd. d. hôp. de Paris **45**:1380, 1921.

17. Huebschmann, P.: Pathologische Anatomie der Tuberkulose, Berlin, Julius Springer, 1928.

cases seemed similar to the liver in early stages of Laennec's cirrhosis. Spring further stated that, contrary to the observations of Roque,[18] ascites and splenic hyperplasia, so typical of Laennec's cirrhosis, are absent in tuberculous cirrhosis. In none of my cases was there ascites or splenic hyperplasia.

Spring suggested, however, that changes in the liver which resemble Laennec's cirrhosis with less pronounced regeneration of bile ducts and liver cells, and unassociated with ascites and splenic hyperplasia, but with tuberculous etiology, should be classified under the term sclerosis of the liver. My investigation leads me to believe that the type of regeneration in these cases is not sufficiently different to warrant making of it a differential diagnostic feature. The presence or absence of ascites and splenic fibrosis seems of greater significance. But I agree that the conditions in these livers should not be classified as Laennec's cirrhosis, and the term sclerosis for this type of changes in the liver seems justified.

As stated before, eighty cases showed miliary tubercles in the liver and fifty-five both proliferation of connective tissue and miliary tubercles. Two of the four cases that were typical for sclerosis, however, revealed no tubercles, even though many blocks were searched carefully, while the two other cases showed very young tubercles. In forty-three cases of fibrosis of the liver and two cases of sclerosis of the liver there were tuberculous ulcers in the intestines.

The cause of the proliferation of connective tissue in the liver is not known. Stoerk [19] stated the belief that the proliferation of connective tissue in guinea-pigs is directly referable to the localization of tubercle bacilli in the region of the capsule of Glisson. Whether or not this hypothesis is true cannot be said. Lavenson and Karsner [4] stated that, in cases in which tubercles are not seen in the liver, the increase in connective tissue may be the result of the action of the products of the tubercle bacilli.

My figures indicate that there is no relationship between the proliferation of connective tissue and the presence of tubercles in the liver. If tubercles are found in "cirrhotic" livers, they may be independent of the connective tissues (Sternberg).[20] As stated before, a proliferation of bile ducts was found to a slight degree only. In tuberculous guinea-pigs, however, the proliferation of bile duct is, according to Stoerk,[19] extremely marked. I was also impressed by the marked new formation of bile ducts in the livers of fifty guinea-pigs which were injected with material containing tubercle bacilli. This proliferation of

18. Roque, G.: Des cirrhose tuberculeuses du foie, Médicine **2**:757, 1921.

19. Stoerk, O.: Ueber experimentelle Leberzirrhose auf tuberkuloeser Basis, Wien. klin. Wchnschr. **20**:847, 1011 and 1048, 1907.

20. Sternberg, C.: Leber, Gallenblase und Gallenwege, Pankreas, in Aschoff: Pathologische Anatomie, Jena, Gustav Fischer, 1919.

bile ducts, however, was found independent of the formation of the tubercles. The tubercles and the tissue reaction are probably caused by two different agents. While the tubercles are caused by the tubercle bacilli, the increase in connective tissue and the proliferation of bile ducts may be caused by the toxic products of the bacilli.

PANCREAS

Tubercles.—Tubercles of the pancreas are rare. The textbooks state that even though the lymph nodes around the pancreas may, to a great extent, be involved in tuberculosis, the pancreas shows no tubercles. The percentage of tuberculosis in the pancreas varies. Kudrewetzky [21] reported tubercles in the pancreas in 9.37 per cent of his 129 cases of tuberculosis of the lungs. Van Valzah [22] found only one case among 200. This variation probably depends on the number of sections examined microscopically. Robinovitch [23] and his co-workers believed that lipase, or steapsin, and to some extent insulin, may be responsible for the apparent resistance of the pancreas to the formation of tubercles. In my series of 100 cases I encountered tubercles in the pancreas four times, but in none of my cases did I observe an extension of tuberculous lymph nodes into the pancreas, even though in five cases the neighboring nodes were filled with caseous material.

Connective Tissue.—Increase in connective tissue in the pancreas in cases of chronic tuberculosis has not received much attention in the literature, even though it seems a rather frequent observation. The textbooks, too, hardly mention this. Italia [24] believed that while the pancreatic cells destroy the tubercle bacilli, the products of the tubercle bacilli produce an increase in connective tissue. Gilbert and Weil [25] found in thirteen of their twenty-five cases a so-called sclerosis of the pancreas. Opie [26] described two cases of advanced tuberculosis in which a chronic interstitial pancreatitis was found, but only one of these cases showed tubercles in the pancreas. In three cases with tubercles in the pancreas, no proliferation of connective tissue was observed. Lavenson

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21. Kudrewetzky: Ueber Tuberkulose des Pankreas, Ztschr. f. Heilk., vol. 13; abstr., Centralbl. f. allg. Path. u. path. Anat. **3**:1011, 1892.

22. Van Valzah, S. L.: Tuberculosis of the Pancreas, Am. Rev. Tuberc. **9**: 409, 1924.

23. Robinovitch, L. G.; Stiles, G. W., and Payne, E. F.: The Pancreas and Tuberculosis, Endocrinology **9**:490, 1925.

24. Italia, F. E.: Pancreas und Tuberculose, Riforma med., vol. 1, p. 55; abstr., Centralbl. f. allg. Path. **14**:702, 1903.

25. Gilbert, A., and Weil, P. E.: Étude anatomo-pathologique comparative de tuberculose du foie et du pancréas, Arch. de méd. expér. et d'anat. path. **14**: 729, 1902.

26. Opie, E. L.: Cytology of Pancreas in Cowdry, E. V.: Special Cytology, New York, Paul B. Hoeber, 1928.

and Karsner [4] mentioned fibrosis of the pancreas as occurring in two cases, and chronic interstitial pancreatitis as occurring in nine in their series of fifty cases of tuberculosis. Van Valzah [22] was of the opinion that tuberculous or toxic sclerosis of the pancreas in cases of tuberculosis is a rather frequent occurrence, but is not characteristic of tuberculosis. Scholtz,[27] however, did not mention tuberculosis as of etiologic moment in cirrhosis of the pancreas. Kirch [10] believed that tuberculosis may produce a productive chronic inflammation of the pancreas which, theoretically, at least, could precede cirrhotic changes, similar to those found in the salivary glands. In his opinion, these changes are analogous to the observations in the livers of tuberculous patients.

In none of my cases could the diagnosis of fibrosis of the pancreas be made grossly. Histologically, the diagnosis was based on the finding of an increase in connective tissue in the interlobular spaces. The connective tissue surrounding the ducts was not used as a criterion of increase. Eleven cases of my series of 100 showed a definite proliferation of connective tissue. In nine of these cases, the connective tissue extended only to a varying degree between the lobules, but did not encircle the lobules and showed no intra-acinar proliferation. Between the fibers of connective tissue, a few lymphocytes and endothelial cells were demonstrable. None of these nine cases showed tubercles in the pancreas. The remaining two offered a marked new formation of connective tissue encircling the lobules entirely. In some instances, the connective tissue extended between the acini and separated individual cells. Some of the acini were extremely small and consisted of cells with deeply stained nuclei. There was a slight new formation of ductules present, which in some sections were seen as islets in the midst of connective tissue. The connective tissue was infiltrated by a varying number of lymphocytes and endothelial cells. The islets of Langerhans seemed to be spared by the fibrotic process. Some of the sections showed as many as eight islets in a field. This observation may be explained by the retraction of the surrounding connective tissue, drawing the islets closer together. Only one of these two cases showed, in addition, tubercles, but these tubercles were not surrounded by connective tissue, and no relation between the tubercles and the proliferation of connective tissue could be made out. It may be especially emphasized that none of the cases was the seat of obstruction of the pancreatic duct.

Scholtz [27] called sclerosis of the pancreas combined with regeneration, cirrhosis of the pancreas. While, in his opinion, in accord with Poggenpohl,[28] some increase in connective tissue of the pancreas in cases of cir-

27. Scholtz, D.: Beiträge zur Pankreaspathologie, Virchows Arch. f. path. Anat. **247**:467, 1923.

28. Poggenpohl, S. M.: Zur Frage der Veraenderungen des Pankreas in Lebercirrhose, Virchows Arch. f. path. Anat. **196**:466, 1909.

rhosis of the liver is not uncommon, yet pancreatic cirrhosis is a rare observation. Nine of my 100 cases showed an increase in connective tissue in the pancreas, while two showed the pancreas to be the seat of a marked new formation of connective tissue combined with some manifestations of regeneration. According to Scholtz,[27] these two cases should be called cirrhosis of the pancreas. Similar changes were encountered in the liver, but, as was brought forward in the discussion of fibrosis of the liver one is not dealing in such a case with a true cirrhosis of the liver, but with a condition that was called sclerosis. The amount of newly formed connective tissue in the pancreas in these two cases was great, but the relatively small amount of regeneration made the term sclerosis of the pancreas seem more appropriate than cirrhosis.

Table 2 is presented to show the changes in the liver in the cases of

TABLE 2.—*Summary of Observations in Cases of Fibrosis of the Pancreas*

		Liver			Pancreas			Tuberculous Ulcers of Intestines
Case	Age	Connective Tissue	Portion Infiltrated by Fat	Tubercles	Fibrosis	Sclerosis	Tubercles	
2	27	Increase	⅛ *	Present	Present	.....	Absent	Present
23	29	Increase	⅛	Absent	Present	.....	Absent	Present
31	48	Increase	Absent	Present	Present	.....	Absent	Present
32	30	Increase	⅛	Present	Present	.....	Absent	Present
45	60	Increase	Periportal spaces	Present	.....	Present	Present	Present
33	46	Increase	⅛	Present	Present	.....	Absent	Absent
47	40	Absent	Periportal spaces	Present	Present	.....	Absent	Present
50	16	Increase	Absent	Present	Present	.....	Absent	Present
55	54	Increase	Absent	Present	Present	.....	Absent	Absent
95	24	Absent	⅛	Absent	.....	Present	Absent	Absent
100	37	Sclerosis	Absent	Absent	Present	.....	Absent	Present

* ⅛ of the liver lobule.

fibrosis of the pancreas. It further gives the age of the patient, and states whether or not tuberculous lesions were found in the pancreas and intestines.

There is no relation between fibrosis or sclerosis of the liver and the pancreas in these cases, or between the age of the patient and the fibrotic changes in the pancreas. Otherwise these observations do not justify further conclusions.

Islets of Langerhans.—Before this study was undertaken, it was observed in routine examinations of the pancreas in cases of tuberculosis that the islets were much larger in some cases than in others. In the course of the histologic investigation, therefore, special attention was given to the size of the islets. No microscopic changes in the islets were noted in my cases. The cells appeared uniform in size and were sharply defined. No cellular infiltration and no degeneration could be made out.

Size of Islets.—The size of the islets in the normal pancreas varies greatly. Laguess [29] classified the islets according to their size. He differentiated very small islets (less than 100 microns in diameter), small (from 100 to 150 microns), medium (from 150 to 200), large (over 200) and giant islets (over 400). He believed that giant islets were exceedingly rare. The largest diameters of the islets recorded in five cases by Dewitt [30] were 380, 350, 330, 325 and 320 microns. MacCallum [31] found the normal average diameter to be 157 by 146 microns. Among ninety cases of diabetes, Cecil [32] found 38 per cent with islets measuring 400 microns in diameter, or more, but he believed that these islets were hypertrophied. Heiberg [33] held that the largest diameters of the islets varied from 225 to 275 microns. In cases of diabetes, he found hypertrophic islets measuring 400 microns in diameter. Gray and Feemster [34] gave the largest diameter in a new-born child as 206 microns. In a child born from a diabetic mother, they observed hypertrophic islets, the largest of which measured 728 by 324 microns. Dubreuil and Anderodias [35] described a similar case with the largest islet measuring 394 by 335 microns. Wright [36] called diameters of from 250 to 300 microns the normal limit; however, he gave the largest diameters as 411, 465, 520 and 602 microns. But islets of all his cases showed hyaline degenerative changes.

I readily recognize the difficulties in establishing the normal sizes or diameters of the islets. As long as the whole pancreas is not examined histologically and every one of the islets in the section is measured, no exact statements can be made as to the size of the islets. According to the figures mentioned, however, it is fairly safe to assume that the average largest diameter of normal islets in man measures around 300 microns.

As mentioned previously, the diameters of a number of islets from the region of the head, midportion and tail of the pancreas were

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29. Laguess, E.: Compt. rend. Soc. de biol. **9**:402; 1893; **10**:667, 1894; cited by Cecill (footnote 32.

30. Dewitt, L. M.: Morphology and Physiology of Areas of Langerhans in Some Vertebrates, J. Exper. Med. **8**:193, 1906.

31. MacCallum, W. G.: Hypertrophy of the Islands of Langerhaus in Diabetes Mellitus, Am. J. M. Sc. **133**:432, 1907.

32. Cecil, R. L.: A Study of the Pathological Anatomy of the Pancreas in Ninety Cases of Diabetes Mellitus, J. Exper. Med. **11**:266, 1909.

33. Heiberg, K. A.: Studien ueber die pathologisch anatomische Grundlage des Diabetes Mellitus, Virchows Arch. f. path. Anat. **204**:175, 1911.

34. Gray, S. H., and Feemster, L. C.: Compensatory Hypertrophy and Hyperplasia of the Islands of Langerhans in the Pancreas of a Child Born from a Diabetic Mother, Arch. Path. **1**:348 (March) 1926.

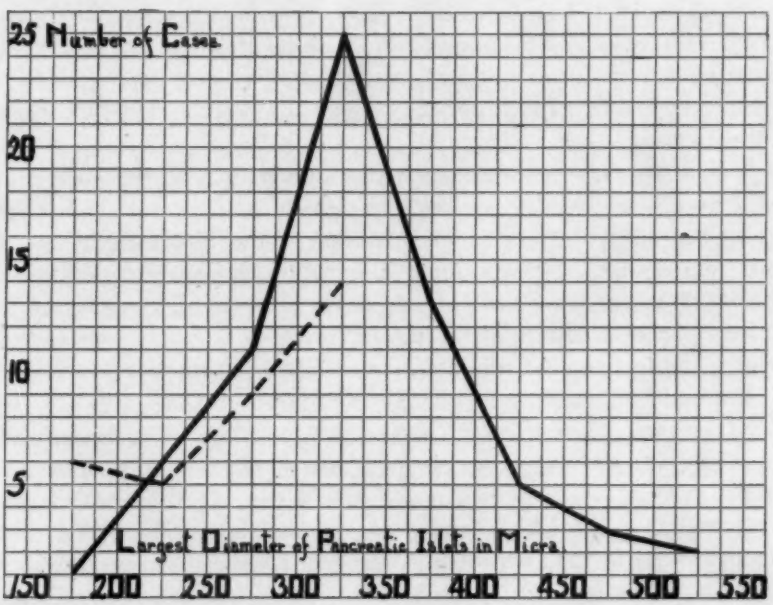
35. Dubreuil, G., and Anderodias: Illots de Langerhans geants chez un nouveaux né, issue de mère glycosurique, Compt. rend. Soc. de biol. **72**:1490, 1920.

36. Wright, A. W.: Hyaline Degeneration of the Islands of Langerhans in non Diabetics, Am. J. Path. **3**:461, 1927.

measured with a filar ocular, and the largest diameter of each case recorded. An attempt to measure the smallest diameter was given up because of the great variation at the levels in which the islets were cut. Small diameters may mean not really small diameters of the islets but diameters of segments of the islets. If the diameters are large, however, it follows that the islets are large.

TABLE 3.—*Largest Diameters of Islets of Langerhans in Relation to Presence and Absence of Fat in the Liver*

Measurement of Islets, Microns	Fat in Liver Present, Number of Cases	Fat in Liver Absent, Number of Cases
150-199	6	1
200-249	5	6
250-299	9	11
300-349	14	25
350-399	..	13
400-449	..	5
450-499	..	3
500-549	..	2



Graph of incidence of fat in liver, and size of pancreatic islets. Continuous line indicates cases without fat in liver. Interrupted line indicates cases with fat in liver.

The largest diameters of the islets in my cases varied from 155 to 511 microns. In comparing the slides of the pancreases with those of the livers, it was noted that cases with fat in the liver showed smaller islets than those without fat. Table 3 summarizes the numbers and measurements of the islets of cases with and without fat in the liver. The accompanying chart shows these figures graphically.

Fourteen cases with fat and twenty-five without fat in the liver showed islets measuring from 300 to 349 microns in diameter. None of the islets in cases with fat in the liver measured over 350 microns, but twenty-three cases without fat in the liver showed islets which measured more than this. "Giant" islets were found in the pancreas in ten cases without fat in the liver, and none in the pancreas in cases with fat in the liver. In my cases of complete infiltration of the liver by fat, the largest islets did not exceed 275 microns in diameter. The islets of cases showing fat in the liver in about half of the lobule did not exceed 300 microns in diameter.

The presence or absence of connective tissue or even of sclerosis of the pancreas, in my cases, seemed to have no influence on the size of the islets. Opie [37] believed that hypertrophy of the islets had been observed in association with lesions that destroy some of these structures in man. But it may be especially emphasized that none of my cases showed clinical signs of diabetes. Only one reference was found to hypertrophy of the islets in tuberculosis. Kasarnowskaja [38] stated that hypertrophy of the islets of Langerhans is found in cases of tuberculous toxemia of long duration. But he did not give any details as to the measurements of the islets, nor did he give any description of the organs in these cases.

It is, of course, questionable whether hypertrophy of the islets necessarily means an increase in function, which would result in hypoglycemia; but unfortunately an examination of the blood sugar was not made in my cases. It was interesting in this connection that of 131 patients suffering from chronic pulmonary tuberculosis, twenty, or 15.3 per cent, showed less than 80 mg. of sugar per hundred cubic centimeters. Folin's method was used for the blood sugar determinations.

Macleod [39] showed that after pancreatectomy in dogs the liver contained excessive quantities of fat, which promptly disappeared after administration of insulin. Campbell and Macleod [40] maintained that insulin reduces the fat content of the liver. Assuming, then, that hypertrophy of the islets implies an increase in function, one would expect less fat in the liver in cases showing larger islets. It was shown before that the liver contained fat only in cases in which the diameter of the islets was less than 350 microns, and that three cases of complete

37. Opie, E. L.: Disease of the Pancreas, Philadelphia, J. B. Lippincott Company, 1903.

38. Kasarnowskaja, O. S.: Ueber die Veraenderungen im endokrinen Apparat der Bauchspeicheldruese unter dem Einfluss tuberkuloeser Toxaemia, Beitr. z. Klin. d. Tuberk. **65**:777, 1927.

39. Macleod, J. J. R.: Insulin, Eleventh Internat. Physiol. Cong., July 24, 1923; Lancet **205**:198, 1923.

40. Campbell, W. R., and Macleod, J. J. R.: Insulin, Medicine **3**:195, 1924.

infiltration of the liver by fat showed islets in the pancreas not exceeding 275 microns in diameter. Thus, in some cases of chronic tuberculosis, the islets of Langerhans were larger than normal, and such cases did not show fat in the liver. The infiltration of the liver by fat in these cases may have depended on the size of the islets of Langerhans as well as on other factors.

Abraham [41] and Rosenberg and Wolf [42] found that tuberculosis may produce a lowered tolerance for insulin, leading in advanced cases to sudden hypoglycemia. It is possible that hypertrophy of the islets, in these cases, may be responsible for the sudden development of hypoglycemia.

SUMMARY

Among 100 cases of chronic ulcerative tuberculosis of the lung, thirty-four showed the presence of fat in the liver. Only three of these cases showed complete infiltration of the liver by fat. There was no relation between the infiltration of the liver by fat and the presence of tubercles in the liver and ulcerative tuberculous lesions in the intestines. There was no relation between age, color or sex and the infiltration of the liver by fat. Eighty of the cases showed tubercles in the liver. An increase in connective tissue was present in 67 per cent, but only four cases showed changes that were called sclerosis. The pancreas showed tubercles in 4 per cent. In eleven cases an increase in connective tissue was demonstrated. Only two cases showed sclerosis. There was no relation between the fibrotic changes in the liver and those in the pancreas, between tubercles of the pancreas and tuberculous lesions of the intestines, or between fibrotic changes of the pancreas and the age.

Special attention was given to the size of the islets of Langerhans. In cases in which fat was found in the liver, the largest diameter of the islets measured less than 350 microns. The diameters did not exceed 275 microns in cases of complete infiltration of the liver by fat. The pancreas in twenty-three cases which showed no fat in the liver had islets measuring more than 350 microns in diameter; ten of these islets measured over 400 microns. On the assumption that larger islets mean increased function, which, of course, is questionable, it was suggested that, it may depend on the size of the islets of Langerhans, together with other factors, whether or not the liver in cases of pulmonary tuberculosis contains fat. The larger islets may cause the sudden hypoglycemia found in some cases of diabetes complicated with tuberculosis during the course of treatment with insulin.

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41. Abraham, A.: Ueber die Lungentuberkulose der Diabetiker und ihre Behandlung mit Insulin und Synthalin, Med. Klin. **23**:720, 1927.

42. Rosenberg, M., and Wolf, G.: Diabetes, Lungentuberkulose und Insulin, Klin. Wchnschr. **6**:936, 1927.

Laboratory Methods and Technical Notes

A PARAFFIN METHOD FOR SECTIONS WITHOUT ETHYL ALCOHOL*

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Owing to difficulty in obtaining suitable ethyl alcohol, a routine rapid method for preparing tissues for section without ethyl alcohol has been evolved. In results, this method compares favorably with any of the longer routines. Specimens are preserved in a diluted solution of formaldehyde, U.S.P. (1:10) until ready to dehydrate.

FIXING

The fixative consists of: absolute methyl alcohol, 6 parts; chloroform, 3 parts, and glacial acetic acid, 1 part.

The fixative must be freshly prepared. Use small, wide-mouth bottles of 50 cc. capacity, and about 30 cc. of fixative for each bottle. When several pieces of tissue from the same case are to be examined, use larger amounts of fixative in proportion.

1. Cut pieces 3 by 6 mm. and place them in the fixative from ½ to 3 hours, according to the size of the block.
2. Pour off the fixative and add absolute methyl alcohol.....1 hour
3. Absolute methyl alcohol.....1 hour
4. Blot (by placing tissue on filter paper).
5. Chloroform and paraffin (saturated) at 38 C.....6 hours or overnight
6. Paraffin (56 C.).....2 hours
7. Block and cool quickly.
8. Cut sections from 6 to 8 microns thick.
9. Fix sections to slides with the usual albumin fixative.

STAINING

1. Xylene .....3 minutes
2. Absolute methyl alcohol.....3 minutes
3. 70 per cent methyl alcohol.....3 minutes
4. Tap water .....3 minutes
5. Harris hematoxylin .....6 minutes
6. Acid alcohol (1 per cent hydrochloric acid in 70 per cent methyl alcohol) .....10 seconds
7. Ammonia water (8 drops to tumbler of tap water; must be freshly prepared) .....5 minutes
8. Tap water .....8 minutes
9. Eosin (¼ of 1 per cent watery solution).....½ minute
10. 95 per cent methyl alcohol.....on and off
11. Absolute methyl alcohol.....on and off
12. Absolute methyl alcohol.....on and off

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* From the Ellis and Butler Laboratories.

13. Xylene .....on and off
14. Xylene.
15. Mount in xylene balsam.

For those who desire a more rapid method, the following is suggested, but not recommended as routine:

1. Place small pieces of tissue in acetic methyl alcohol fixative.....1 hour
2. Absolute methyl alcohol.....1 hour
3. Absolute methyl alcohol.....1 hour
4. Blot.
5. Xylol .....½ hour
6. Blot.
7. Paraffin (56 C. incubator) .....1 hour
8. Block and cool quickly.
9. Cut sections and stain as described.

SUMMARY

A rapid method for preparing tissue for section without ethyl alcohol has been developed which has proved reliable. Any of the ordinary staining methods may be used following this new method of fixation.

General Review

RECENT WORK ON THE EFFECTS OF INANITION AND OF MALNUTRITION ON GROWTH AND STRUCTURE *

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* Submitted for publication, July 10, 1928.

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Effects of a Deficiency of Vitamin E (Antisterility)
Reproduction in the Male (Sterility)
Reproduction in the Female (Sterility)
Lactation
Paralysis of the Young
Effects of a Deficiency of the Recently Discovered Vitamins
Antipellagra Factor (P-P), B_2 or Vitamin F
Other Vitamins (Vitamin G)

The term inanition as used here in its broader sense indicates a pathologic condition of body due to the lack of any foodstuff (including water) which is essential to the living organism. As to the character of the inanition, one may distinguish total inanition, with absence or insufficiency of all forms of nutriment, from partial inanition, with the absence or insufficiency of one or more but not all of the various essential constituents of food. As to the degree of inanition, one recognizes (1) complete inanition, with entire absence of all food (as in total inanition) or of the deficient elements (as in partial inanition), and (2) incomplete inanition, with merely an insufficiency of either all food or certain essential elements.

The various types of inanition are summarized in the following tabulation:

- Inanition
  - A. Total (quantitative)
    - 1. Complete—no food or water
    - 2. Incomplete—general underfeeding
  - B. Partial (qualitative)
    - 1. Complete (entire absence)
      - Of one or more of the necessary foodstuffs
        - Proteins
        - Fats
        - Carbohydrates
        - Salts
        - Vitamins
        - Water
    - 2. Incomplete (insufficient amount)

The subject of inanition is accordingly broad in scope and includes various disorders arising from deficiencies of essential foods. Even when restricted to the morphologic phases, the bibliography to 1924 included about 2,700 titles, which were reviewed in my previous work (Jackson, 1925). The present survey of the more recent literature (including also a few papers previously overlooked) comprises about 730 titles, which will be classified according to the various deficiencies and the organs affected. It is hoped that a systematic review of this literature, even though necessarily somewhat brief and superficial, will be useful to numerous workers in the field of nutrition. The point of view in this survey is primarily morphologic. The physiologic and biochemical aspects are therefore mentioned only incidentally in relation to the effects on growth and structure.

The physiologic and chemical aspects of inanition are fully considered, and an extensive bibliography is furnished, in the treatises by Aron (1924) and McCollum and Simmonds (1925). Reviews of the nutritional disorders, especially from the physiologic and clinical standpoints, were contributed by Burnett (1925, 1926, 1927). Elias (1925)

discussed the general relation of inanition to pathology and medicine recalling the dictum of Hippocrates that hunger has "potentia sanandi, debilitandi et occidendi." He reviewed the effects of starvation on the weight, on the blood-vascular system and on the urine, together with its therapeutic applications in various diseases.

TOTAL INANITION

Under the head of total inanition will be considered the effects of total inanition in man and lower organisms. Since the effects of subsistence on water alone are similar to those in total inanition, they will also be considered here, although, according to a strict definition, this state is a form of partial inanition.

EFFECTS ON THE BODY IN GENERAL

The studies of total inanition in human beings have to do almost entirely with incomplete inanition: i.e., with the effects of a general underfeeding of various grades. In conditions of malnutrition or famine in man, however, there is great variation in the extent to which the various essential factors of nutrition are deficient. This complicates the results, which are correspondingly variable and difficult of interpretation. Some of the effects of famine will therefore be discussed later under the head of partial deficiencies, such as malnutritional edema (due to a deficiency of protein), scurvy (due to a deficiency of vitamin C) and rickets (due to a deficiency of vitamin D).

Attention may be called first to several works which involve a more or less extensive review of various phases of the subject. The publications of Rössle (1923), Schlesinger (1925) and Czerny and Keller (1925) included observations on the effects of inanition and malnutrition in children, with extensive bibliographies. Variot (1925) summarized the previous work by himself and his associates, including that on the dissociation of ponderal and statural growth under various conditions of malnutrition. Herzenberg (1926) and Stefko (1927) reviewed the effects of the Russian famine. An unusually severe type of tuberculosis in malnourished Russian children was described by Stefko (1923). The variations in effect on the weights of organs according to the type of acute or chronic malnutrition in children were discussed by Aron and Pogorschelsky (1926). Edelmann and Saxl (1922) described a characteristic syndrome with cachexia and general glandular insufficiency that may appear as a result of inanition or infections. Nearly a century ago, Howard (1839) gave an extensive account of the clinical symptoms and postmortem appearances caused by underfeeding among the industrial population of Manchester. The increased susceptibility to infections was recognized. The brain and meninges were found congested; viscera generally atrophied and anemic; blood scanty and pale;

heart flabby; stomach and intestines empty and contracted, and the gall-bladder invariably distended with bile. None of these observations, however, was considered specific for starvation.

General Effects on Children.—Indexes of Nutrition: There is continued dispute as to the significance of various physical indexes in the diagnosis of malnutrition in children. Many clinical observers, for example, Jaenicke (1921) and Vonessen (1921), found that Rohrer's ratio of weight to height and similar indexes of bodily build are inadequate as indications of the nutritional condition, unless supplemented by additional clinical data.

Hereditary and racial differences in build also must be considered, especially where mixed populations occur. Dublin and Gebhart (1924) found the usual American tables of heights and weights inapplicable to children of Italian parentage in New York City. Emerson and Manny (1924, 1924a), however, using the new standard tables of the National Health Council, claimed that children 7 per cent or more underweight (for height) invariably manifest other unmistakable signs of malnutrition. Similarly, Garrahan and Bettinotti (1924) concluded that Wood's tables are as reliable as any of the more complicated methods in detecting the undernourished children in Buenos Aires.

Guttmann (1925) devised a new functional test (based on the expansion of the circumferences of the elbow and the chest) which is said to agree closely with the clinical diagnosis of malnutrition, whereas the Rohrer index gives agreement in only 29.6 per cent of the cases, the Bornhardt index in 30 per cent, the Livi index in 32.5 per cent and the Brugsch index in 44.6 per cent. Tsurumi and Nakatate (1924) asserted that Pirquet's index of nutrition cannot be applied to the Japanese. Beeuwkes (1926) likewise found Pirquet's "Pelidisi" system unreliable as an index of nutrition in the Russian famine, especially in cases of rickets and hunger edema. The various physical indexes of nutrition were discussed in detail by Paton and Findlay (1926). The work of Variot and his students on the dissociation of statural and ponderal growth was confirmed by Troncay (1923), and reviewed by Variot (1925).

Effects on Weight at Birth and the Ratio of the Male to the Female Sex: The data cited in my previous work (Jackson, 1925) indicated that in most cases there had not been significant decreases in weights at birth in Europe on account of the food shortage during the war. Binz (1919) added records from 8,000 births in Munich showing that changes had not appeared in the average length of body, but that a slight decrease had appeared in weight at birth and in circumference of head in 1917, compared with 1914. Bondi (1924) found in Vienna a definite decrease in the average weight at birth from 3,201 Gm. in 1913 to 3,023 Gm. in 1916. Abels (1925a) likewise showed that the average weight at birth

in Vienna was lower during the period from 1919 to 1922 than in the subsequent two years with better nutrition; also that the monthly average reached a maximum of from 3,300 to 3,350 Gm. in the summer, decreasing to a minimum of about 3,100 Gm. during the winter (on account of a deficiency of vitamins). Sorokin (1925) reported a considerable decrease in Russian weight at birth, and an increase in the percentage of stillborn infants, and deaths of infants shortly after birth. Slemons and Fagan (1927) somewhat doubtfully concluded that overgrowth of the fetus can be prevented by underfeeding during pregnancy.

According to Bayer (1924), the Bavarian statistics show a fairly constant prewar ratio of 1,062 males to 1,000 females. The first term of this ratio rose to 1,087 in 1918, decreasing again to 1,072 in 1922. He doubted whether inanition, however, was responsible for the change. The consideration of effects of famine in war time on the number of births, will be included later under the head of the effects on the reproductive system (ovary).

Retardation of Growth: The incidence of malnutrition among infants and older children as a result of the conditions of war likewise appears somewhat variable. Thiele (1917), of Chemnitz, concluded that, to 1917, the health of German school children in the country districts was unimpaired by malnutrition, but that in the large cities an increasing amount of retardation in physical development was evident. Bachauer (1917), of Augsburg, did not find any change for the year 1917; but for 1921 he noted a decrease in average weight and also in the range of variation. Haeberlin (1918), in a review of the literature, did not find any detrimental effect of conditions due to the war on weight or length of body, girth of chest, etc., in German children. Birk (1918), of Kiel, did not note any bad effects on children of preschool age, but observed the effects of malnutrition among school children. Stephani (1923) did not find any significant changes in the average stature and weight of Mannheim school children on comparing the measurements for the corresponding ages in the prewar years (1912 to 1914), the war years (1916 to 1918) and the postwar years.

In Vienna, however, Zappert (1920) noted among infants of the first year a marked decrease in average weight during 1916 and 1917, which reached a minimum in 1918, with a rapid increase following in 1919. Kaup (1921), of Munich, also found a decrease of from 1.2 to 2 per cent in the average stature and of from 3.2 to 5 per cent in the average weight of 1,200 "Berufsrekruten" in 1920, in comparison with the corresponding data for 1913. Davidsohn (1919, 1922), in Berlin, Jaenicke (1921), in Apolda, Vonessen (1921), in Köln, Schlesinger (1922, 1924), in Frankfurt, and Schmidt (1924), in Bonn, likewise found in school children a subnormal weight and stature, which was ascribed to previous malnutrition during the war. Stefko (1925),

Sorokin (1925) and Newsoroff (1927) cited data similarly indicating that the Russian children still showed marked subnormality in average weight and length on account of the previous period of war and famine.

Recovery from Effects of Malnutrition: Children show great variability in their capacity for recuperation, depending largely on the duration and character of the previous malnutrition. Bloch (1920) observed a marked increase in stature and weight among thirty malnourished girls taken from Halle to a sanatorium in Switzerland for six weeks. Schlesinger (1922, 1924) made an especially careful statistical study of the subject among school children in Frankfort-on-the Main. Retardation increased progressively during the war and apparently persisted thereafter. The greatest retardation in stature in most age groups occurred in 1920, the average stature being 4.3 per cent below normal. There was great improvement in 1921-1922, the average stature approaching normal in many groups, but a marked decline again in 1923. The maximal retardation in weight was reached in 1917, with a subnormality of from 4 to 12 per cent in the various groups (the greatest deficiency occurring at the age of 3 years). In the following years there was improvement, with a decrease again in 1923. Rohrer's index did not always vary in correspondence with the changes in average weight. Blühdorn and Lohmann (1922) followed thirty-eight cases of infantile malnutrition. Of these ten resulted in death, but most of the others eventuated in full recovery of the normal length and weight of body. Jenks (1926) presented data indicating that school children of Munich were still subnormal in weight and stature. Roberts (1927) concluded from repeated examinations that American children underweight or overweight tend to remain so. According to Variot (1925), the chances for recovery under proper refeeding are good in most cases, even after an extensive period of repression of growth. Stefko, on the other hand, concluded that the prolonged famine had reacted unfavorably on the general constitution of the Russian people.

Gribbon (1922), in Vienna, and Paton and Findlay (1926), in Scotland, found little evidence that malnutrition in children is caused directly by lack of nourishment. But Paterson and Marr-Geddes (1927), from a study of 100 cases, concluded that the chief cause of gastric trouble and failure to gain in weight among the outpatient infants of hospitals was underfeeding or starvation.

The extent of the endurance of total inanition in the new-born is indicated approximately by the length of life of infants born with congenital atresia of the esophagus. Sheldon (1926) reported six cases in which the duration of life ranged from six to thirteen days, averaging nine days.

Effects on Vertebrates.—Adult Animals: A comprehensive review of the effects of inanition on animals was given by Aron (1924). The effects on behavior in animals were reviewed by Stone and Lindley (1928).

The question as to the effect of inanition on tumors has often been investigated, with somewhat variable and inconclusive results. Sugiura and Benedict (1926) found that transplants of the Flexner-Jobling rat carcinoma disappeared in 73 per cent of the underfed hosts, and in only 18 per cent of the fullfed controls. However, underfeeding after the engrafted cancerous tumors had been well established did not prevent subsequent growth of the tumors. The results confirmed the observations of Moreschi (1909). Favorable results were also obtained by underfeeding mice that had spontaneous mammary carcinomas.

A decreased resistance to various types of infection during inanition has frequently been observed in man during famine and also in experiments on animals. Schwarz (1927) demonstrated that intravenous injections of streptococcus and staphylococcus were much more rapidly fatal in starved young mice; weakened cellular reactions to the infection were observed in the spleen and the kidney. The possible relations of a deficiency of vitamins to infection and malignant growths will be considered later.

According to Yamasaki (1923), adult mice on diets productive of total inanition live four or five days, with a loss of about 27 per cent in weight. Danforth (1927), however, found that in a peculiar, markedly obese strain of yellow mice obtained by cross-breeding, a diet of lettuce and water led to a decrease in weight from 73 Gm. to 24 Gm. (a loss of 67 per cent) in twenty-eight days, and that refeeding with a normal diet, brought about a complete recovery from a maximal loss of 69.2 per cent. Wetzel (1925) observed that the loss of weight in adult pigeons receiving water only corresponds to the autocatalytic law. The mean duration of life was 25.2 ± 0.9 days. The loss of weight in thirty days was 51.5 ± 0.6 per cent, reaching a maximum of 53.4 per cent in one bird that survived forty days. Dobreff (1927) noted that the dogfish (*Scyllium*) can survive a fast up to 112 days, with a loss of 33 per cent in weight.

Hayashi (1924b) found that in the rat the organs can be classified into three groups according to their several weight curves during inanition: (1) those the weights of which decrease parallel with the body weight—the pancreas and the parotid and submaxillary glands; (2) those the weights of which decrease more rapidly—the thymus, spleen and liver, and (3) those the weights of which decrease less rapidly—the kidney, ovary, testis, hypophysis, thyroid and heart. The suprarenal gland, he found, did not lose weight; often it gained weight. The detailed effects on the various individual organs will be reviewed later.

Young Animals: Hartwell (1927) found that pregnant rats on a restricted diet usually showed a continued increase in weight, although the number of the young rats and their weights were distinctly decreased. The continued growth in length of body by young rats held each at constant weight by general underfeeding (as well as by feeding on diets variously deficient in part) was demonstrated by Winters, Smith and Mendel (1927). This is in agreement with the results of earlier observers demonstrating the dissociation of statural and ponderal growth during malnutrition in man and various animals. Smith and Bogin (1927) observed in underfed young rats a progressive necrosis and dry gangrene beginning at the tip of the tail and extending forward. The vascular walls appeared somewhat thickened, the cartilage cells necrotic and the muscle fibers hyalinized with pyknotic nuclei.

Some experiments have been made confirming the dissociation of growth in mass and length in frog larvae. Krizenecky (1925) observed that young tadpoles of *Rana fusca* (*temporaria*) sometimes continued development (formation and differentiation of the hind limbs) for a few days without food. In another experiment (1926), tadpoles up to 16 days of age were found continuing to grow in length during inanition, the skeleton being formed at the expense of other parts of the body. Groebbels (1925) noted this persistent growth in length during starvation in tadpoles about 14 mm. long, but noted a decrease when the starvation began in tadpoles from 16.8 to 20.5 mm. long. Hertwig (1924) found that in starved frog tadpoles the central nervous system and sense organs were unusually resistant, although the lens fibers underwent liquefaction. The body musculature, the liver and especially the thymus became remarkably reduced in size. The intestine underwent a remarkable involution and a reduction in size, somewhat like the change during normal metamorphosis.

The effect of intermittent fasting on subsequent growth in tritons was discussed by Krizenecky (1918). Podhradský and Kostomarov (1925) demonstrated a persistent growth of the skeleton and the head of carp starved at 6 weeks of age. The increase was lost again later, and did not appear in carp starved from the age of 3 months on. The increase was in fresh weight, not in dry weight. D'Ancona (1921, 1922, 1926, 1927b) made a series of studies of the eel (*Anguilla*) starved from eighty-one to 657 days with loss of from 21.1 to 61.5 per cent in weight. In young eels (1925, 1926b, 1927a), the loss in weight reached 59.6 per cent in five months.

Effects on Invertebrates and Plants.—The effects of inanition in invertebrates and plants were discussed fully in my previous work (Jackson, 1925). The additional papers will be mentioned briefly. For the Protozoa, Vieweger (1918, 1925) studied the structural and volumetric changes in the cell body and nucleus of *Colpidium;* Sokoloff (1923), the effects

in *Bursaria* and *Dileptus*, including the regeneration of amputated portions; Cleveland (1925, 1925a), the effects on several parasitic protozoa (*Trichonympha, Leidyopsis, Trichomonas, Streblomastix*) during starvation of the termite host (*Termopsis*). The remarkable reduction process exhibited by the coelenterates was described by Hadzi (1912), for *Chrysaora*, and by de Beer and Huxley (1924) for *Aurelia*. The changes in *Planaria* were studied by Child (1914) and Willier, Hyman and Rifenburgh (1925). Dawydoff (1924) obtained complete recovery of the Nemertean worm *Lineus lacteus* after reducing it to an embryonal state by inanition. Cardot (1924) observed abnormalities in ovulation and embryonic development caused by malnutrition in the land snail, *Agriolimax agrestris*.

Several studies concerned the effects of starvation on insects, especially during development. These include the experiments of Singh-Pruthi on the mealworm (*Tenebrio molitor*); of Ezikov (1922), Jezhikov (1925) and Cousin (1926) on the blow-fly, *Calliphora;* of Titschack (1926) on the clothes-moth, *Tineola;* and of Stscherbinovskij (1924) on the moth, *Malacosoma*. Some observations on starvation of adult insects were made by Fink (1925) on the potato beetle, *Lepinotarsa*, and by Abbott (1926) on the roach, *Periplaneta*.

Huxley (1921) studied the relative resistance of the various tissues in the process of dedifferentiation during starvation in the Ascidian, *Perophera*. Some further data from experiments on invertebrates will be mentioned later in connection with considerations of the various organs and also under the head of mineral deficiencies.

Two important experiments on plants may be cited. Harris (1912) made an extensive biometric study to determine whether hereditary effects are transmitted in the bean plant (*Phaseolus vulgaris*) after starvation through three generations. Only slight effects persisted after the plants returned to normal nutrition. Schaffner (1925) demonstrated that sex in the higher plants (*Arisaema, Cannabis, Thalictrum, Humulus*) is not predetermined, but can be experimentally modified or reversed by variation in the nutrition. Further studies on plants will be mentioned later in connection with the studies of deficiencies of water and minerals.

EFFECTS ON INDIVIDUAL ORGANS AND SYSTEMS

Integument.—Differences in the relative loss of subcutaneous fat in various regions of the body during malnutrition were reviewed in my previous work (Jackson, 1925). Merkel (1890) noted that fat never disappears from the hairy scalp, even in extreme emaciation. Coerper (1924) emphasized the close relation between the bodily habitus and the amount of subcutaneous fat. The cutaneous turgor and blood supply are indexes of nutrition that are not closely related to the habitus. Ponomareff (1921), as did many other observers, reported an enormous

increase in the incidence of furunculosis and other suppurative lesions of the skin during the Russian famine. The apparent cause was general debility and poor cutaneous circulation (due to weakened heart action). Anitschkoff and Sawodski (1922), in addition, noted a slow regeneration of wounds under such conditions. Engelking (1923) observed the occurrence of an exudative diathesis in younger malnourished children, and of a seborrheal diathesis in older malnourished children.

According to Schulmann and Marek (1927), subcutaneous bullae experimentally produced in rabbits persisted for a much longer period during starvation. Sheldon (1924) described in detail the structure of the so called "hibernating gland" in white rats as it appeared during starvation and refeeding. The changes were similar to those in ordinary adipose tissue. Morpurgo (1927) found it possible to make successful homoplastic transplants of skin in underfed rats, although such transplants invariably failed in normal rats. He believed that the failure was due to a defensive reaction of the host (like resistance to infections), which is reduced by inanition. Chang (1926) noted in underfed albino rats a retardation in the growth of hair, which was improved by thyroid feeding. Ogneff (1908) reported that the lighter color of the starved amphibian, *Axolotl*, is caused by an atrophy of the dark chromatophore cells, which are in part destroyed by phagocytosis. According to Svoboda (1924), the process of ecdysis (desquamation of the skin) in amphibians (*Triton*) depends on the opposing factors of keratinization and formation of nuclear chromatin in the epidermal cells. The former is favored by inanition, and accelerates ecdysis; while the latter is favored by good nutrition and retards ecdysis.

Skeleton and Teeth.—A variable degree of "Hungerosteopathie," involving osteoporosis and osteomalacia, has often been observed in persons subjected to famine or chronic underfeeding. The recent studies of this effect of malnutrition in human beings are chiefly from Russian sources. Stefko (1925a) noted a disturbance of ossification of fetuses from starved mothers. The changes are ascribed to characteristic lesions in the cartilage (chondrodystrophia foetalis ex inanitione). The osseous lesions somewhat resemble those of osteogenesis imperfecta and infantile scurvy (Barlow's disease).

Atrophy and deformities of the vertebrae were likewise found in adults by Stefko (1926, 1927) and Stefko and Schneider (1928). The result of such atrophy is a decrease in stature. Henseler (1913), confirming the earlier work of Nehring, showed a marked effect of malnutrition on the form of the head in swine. Data were cited by Iwanowsky (1923, 1925) and Basler (1925) indicating that even the human adult cranium may become decreased in weight and size, with relative elongation (dolichocephaly), as a result of famine. The result

was ascribed to changes in the bone, and not merely to resorption of the soft parts. Stefko (1927a), however, concluded that the tendency is rather toward brachycephaly, especially in the young (probably caused chiefly by rickets).

Watanabe (1924) found that regeneration of the skull bone after experimental aseptic fracture was not appreciably affected by underfeeding in the guinea-pig and white rat. The effect of a deficiency of vitamins will be mentioned later.

The teeth appear to be relatively resistant to simple inanition or underfeeding. Although it is generally believed that carious teeth are frequently associated with malnutrition in children, Emerson (1925) did not find any correlation between underweight and carious teeth among 1,500 school children in Rochester. However, he stated that orthodontic treatment is not likely to be successful in malnourished children.

Muscular System.—Degenerative changes in the muscles of starved Russian children and adults were noted by Stefko (1927a). Fetuses, during maternal starvation, also presented a general hypoplasia of the muscular system.

According to Corti and Fussi (1919), glycogen droplets disappeared from the sarcoplasm of striated muscle fibers of the hedgehog (*Erinaceus europaeus*) during a few days of fasting, but more slowly in hibernation. During the necrosis of the tail observed by Smith and Bogin in underfed rats, the muscle fibers showed hyalinization with nuclear pyknosis. Hertwig (1924) noted marked reduction of the body musculature in starved tadpoles of *Rana fusca*. Berg and Falk (1924) studied the fat changes in the muscle fibers of the frog (*Rana temporaria*) fasting from one to five months. Dakin and Dakin (1925) observed gradual reduction of the musculature in the goldfish during its starvation up to two months. In the striated muscle fibers of the esophagus in the eel (*Anguilla*), D'Ancona (1926a, 1927b) found the sarcolemma, myofibrillae and lines Z and M more resistant to protracted inanition; the other lines disappeared.

Nervous System.—Anitschkoff and Sawodski (1922) interpreted the observed weakness of the various sphincters (anal, vesical and pyloric) in victims of famine as an effect of starvation on the corresponding spinal nerve centers. Beeuwkes (1926) found the nervous symptoms inconstant in these cases. The nervous system is resistant to loss in weight during starvation. Stefko (1927a) gave tables for various ages showing a subnormality of from 2.5 to 14.5 per cent in the weights of the brains of starved children in comparison with the normal children of corresponding ages. For the younger ones, this probably represents chiefly retardation in growth, however, rather than actual loss in weight of the brain. In adults, the apparent loss in

weight of the brain was 9.5 per cent in eight men of from 48 to 50 years; and 23 per cent in five women of from 20 to 25 years. Some brain weights were recorded also by Sedlezky (1924). The microscopic changes that were noted included atrophy and degeneration of the pyramidal cells of the cortex, with a loosening up of the white matter, especially in cases of edema. The observations of Lenz on the chemical changes in the brain were also cited. Aron, Lasch and Pogorschelsky (1925) and Aron and Pogorschelsky (1926) found that in dystrophic infants the brain weight is retarded less than the body weight, which is in accordance with the conclusions of earlier observers. Variot (1925) showed that in children the growth of the brain in general undergoes relatively slight disturbance during malnutrition.

In fasting tadpoles of *Rana fusca*, Hertwig (1924) similarly observed that reduction in the size of the central nervous system and sense organs does not follow malnutrition.

Eyes.—Few recent observations on the human eye during starvation are available. Engelking (1923) and Harman (1925) discussed the condition known as phlyctenular conjunctivitis, which occurs in malnourished children. The disorder is apparently constitutional rather than local in character, and may be (although this is not suggested by the authors) related to the ophthalmic disorders caused by a deficiency of vitamin A.

Heart.—From the records of 4,871 necropsies performed in American hospitals, Bean (1925, 1926) found the average weight of the heart about 10 per cent lower in emaciated than in well nourished patients. In the Russian cases of inanition reported by Stefko (1924, 1927a) there was a loss in weight and volume of the heart, greater in the young than in adults, and greater in females (especially at puberty) than in males. Microscopic examination revealed atrophy of the cardiac muscle. Stefko thought that the atrophy of the heart may be related to that of the gonads and suprarenal glands during inanition. Some heart weights were recorded also by Sedlezky (1924). Levine (1927) concluded from a review of the literature that the heart is more or less involved in every type of malnutrition, and that malnutrition is therefore a pathogenic factor of prime importance in various cardiovascular disorders.

Blood and Blood Formation.—The results of inanition on the blood are exceedingly variable. Bakwin and Rivkin (1924) found an increase in the relative volume of blood in malnourished infants, which is contrary to the earlier observations of Marriott and Perkins. Lichtwitz (1923) concluded that, in general, the blood is relatively resistant to inanition. Lymphocytosis is regular and hydremia frequent, but anemia is not an early symptom. Curschmann (1923) observed somewhat variable effects of inanition on the blood during the war. It was his

conclusion that neither lymphocytosis nor leukopenia is constant or characteristic, although anemia of a secondary type occurs in severe hunger edema.

Much of the recent work refers to the effects of the Russian famine. Beeuwkes (1926) reported an average erythrocyte count of 4,000,000, and counts sometimes as low as 2,500,000, with poikilocytosis; he frequently found leukopenia with a decrease in polymorphonuclears; and hydremia in 30 per cent of the cases. Stefko (1926, 1927a) concluded, from his extensive studies, that starvation, both prenatal and postnatal, has an especially severe effect on the derivatives of the mesoderm (the blood, the vascular system, the skeleton and the muscles). In Moscow, the proportion of anemic school children steadily increased from 9 per cent in 1919 to 74 per cent in 1925. During famine, the blood picture varies greatly. Stefko (1927a) recognized two groups among the children: (1) those with thickening of the blood, and (2) those with thinning of the blood (cases with edema). In the first group, the number of erythrocytes reaches 6,400,000 per cubic millimeter, with a decrease in the content of water in the blood. There is not any marked change in the total or in the differential leukocyte counts. In the second (edemic) group, the number or erythrocytes may sink to 2,500,000, with hydremia. The leukocyte count is increased, especially the number of the lymphocytes and transitional forms, and the number of myelocytes. The modifications of the blood correspond with the changes in the blood-forming organs. The adipose bone marrow of famished adults becomes gelatinous; that of children undergoes a myeloid metamorphosis. The appearance of myelocytes and normoblasts in the liver indicates here, also, a renewal of the primitive blood-forming function. The changes in the blood and in the blood-forming apparatus during inanition were studied also by Tschelozowa.

Gage and Fish (1924) observed that in man the fatty particles (chylomicrons) that appear in the blood plasma after a meal containing fat gradually disappear within from six to ten hours. In a well nourished man, the particles reappear during the characteristic lipemia after about thirty hours of fasting. The chylomicrons are more distinct and numerous in animals with concentrated, fat-containing food (dog, cat, rat) than in those with bulky food containing little fat (cow, goat, sheep, horse, rabbit).

Suzuki (1925) studied the blood and hematopoietic tissues in guinea-pigs starved from four to thirteen days. The erythrocyte count usually increased somewhat, with early lymphopenia and later pseudo-eosinophil leukocytosis. There was, correspondingly, an atrophy of the lymphoid tissue (lymphatic follicles, splenic nodules) but increased granulopoiesis in the myeloid tissue (bone marrow, spleen and lymphoid organs). Streicher and Emmel (1925) and Emmel and other asso-

ciates (1926) found a decrease of 47 per cent in the total leukocyte count, and of from 63 to 74 per cent in small and large lymphocytes, in albino rats starved 108 hours, with a slight relative increase at 132 hours. The neutrophils decreased in number more rapidly to a maximal loss of 48 per cent at thirty-six hours, with a slight rise thereafter. Schaeffer (1925) observed a loss of about 85 per cent in the combined leukocyte and erythrocyte count in the "pumpkinseed" fish (*Eupomotis gibbosus*) during hibernation, but the blood count later returned to normal without feeding in spite of the progressive loss (up to 38 per cent) in weight.

Spleen and Lymph Glands.—Deposits of hemosiderin in the spleen are much greater in chronic than in acute disturbances of nutrition, according to Stephani (1923). On account of marked individual variations, Hellman (1926) was unable to demonstrate a definite decrease in the amount of lymphoid tissue in the spleen during slight or moderate inanition. However, he found that a marked decrease occurred during prolonged starvation. Aron and Pogorschelsky (1926) found the weight of the spleen nearly parallel with that of the body in infants dying from either acute or chronic dystrophy. They reported that the spleen does not show a definite change in water content. According to Stefko (1927a), the spleen appears subnormal in weight in both young and adult persons who are starving, indicating a relatively greater loss of weight in the spleen than in the body as a whole during the starvation. The weights recorded by Sedlezky may also be consulted here. Stefko observed a marked atrophy of the splenic follicles, which often disappear. The blood vessels are distended. The various kinds of splenic cells (including nucleated erythrocytes in infants) are closely packed, and many, especially those of the myeloid type, show degenerative changes (nuclear pyknosis). The trabeculae and reticular tissue become prominent, especially in adults, in whom is seen almost complete atrophy of the splenic pulp.

Schwartz (1927) found the cellular reaction to infections in the lymphoid and myeloid tissues of the spleen markedly decreased by starvation in young mice. According to Dustin (1923), the atrophy of the lymphatic glands during inanition in new-born kittens is apparently due to emigration of the lymphocytes. Atrophy of the lymphoid structures in general during complete or incomplete inanition has been observed by various authors.

Thymus.—All the recent work confirms the previous demonstrations of the susceptibility of the thymus to involution during all types of malnutrition; such involution was shown to have occurred by Ichok (1925), Variot (1925), Aron and his associates (1925, 1926), Stefko (1927a) and Boyd (1927). Stefko (1925a) found the typical atrophy and cell changes in the thymus of fetuses from starved mothers.

Stephani (1923) noted the same changes in the thymus in infants after either acute or chronic dystrophy. Fat was observed persisting to a variable degree in the thymus cells.

In fasting new-born kittens, according to Dustin (1923), pyknoses appear in the small lymphocytes of the thymus during the first day, becoming abundant on the second day, and undergoing disintegration and disappearance in numerous small areas by the third day. Hammar (1924) listed inanition and nutritional disturbances among the general factors causing involution of the thymus. He stated that in the rabbit, after reduction of the thymus to 1 per cent of its original weight by incomplete inanition, complete recovery to normal weight was obtained within fourteen days by refeeding. Günther (1924) found marked reduction in the thymus of fasting tadpoles (*Rana fusca*).

Alimentary Canal.—According to Beeuwkes (1926), the gastro-intestinal symptoms of the victims of the Russian famine were sometimes slight, but dysentery was characteristic. Variot and his associates (1911, 1925) stated that gastric dilatation is not always caused by overfeeding, but may occur also through aerophagy in underfed, atrophic infants, as was previously noted by Lacau-Saint-Guilly (1913). Faehrmann (1925) observed two cases of ileus in extremely emaciated women. Its pathogenesis is explained by the loss of fat in the omentum, which becomes reduced to a network of blood vessels. Intestinal loops slide into the omental openings. The pressure results in vascular obliteration with slow and progressive strangulation of the intestine.

Most of the recent studies concerned the alimentary canal in the lower animals. Busacchi (1916) claimed that in a dog starved thirty-five days the mitochondria of the intestinal epithelial cells were remarkably resistant, although they sometimes temporarily disappeared during refeeding. Watrin (1924), on the contrary, noted a disappearance of the mitochondria in the surface epithelium of the small intestine in starved rats. The parietal cells of the gastric glands became vacuolated.

Extensive studies on the regressive changes in the stomach and the intestine of underfed young albino rats were made by Miller (1927). The changes were most evident in the mucosa, which showed a variable degree of atrophy and degeneration, especially in the surface epithelium. In extreme cases, the villi appeared almost completely disintegrated. Sun (1927, 1927a) found a similar degeneration of the intestinal villi in albino mice starved only twenty-four hours. Apparent regeneration of the villi occurred within ten hours of refeeding, although four or five days were required for the recovery of the normal weight.

Jolly and Saragea (1924) found a loss of from 40 to 60 per cent in the appendix (cecum) of starved rabbits (which also showed a loss in weight of body of 30 per cent). The lymphoid follicles atrophied; the lymphocytes became scarce, with pyknotic nuclei. Many

large phagocytic cells appeared in the connective tissue. On refeeding, almost normal structure was recovered in fifteen days. Simple atrophy with some hyperemia of the gastric mucosa was observed by Guarino (1927) in guinea-pigs and pigeons starved with or without water.

Barchiesi (1924) found extreme atrophy of the mucosa in the esophagus, stomach and intestine of the tortoise (*Emys orbicularis*) fed water alone for from five to twenty-six months. The epithelial cells were reduced from 83 to 97 per cent in volume; the nuclei from 75 to 78 per cent. Refeeding tests showed that recovery was possible after seven months of starvation. Günther (1924) noted great reduction in the size of the intestine in starved tadpoles (*Rana fusca*). Corti (1920, 1920a, 1921, 1922) made a special study of the migratory cells in the intestinal epithelium during inanition in various amphibia and fishes. Yung (1914) found a reduction to one-sixth in the size of the intestinal epithelium in inanition in various amphibia and fishes. The loss was chiefly in the cytoplasm, the nuclei being more resistant. D'Ancona (1921, 1922, 1926, 1926a, 1926b, 1927, 1927b) made several intensive studies on the alimentary canal of the eel (*Anguilla*) starved up to 657 days, with a loss of 61.5 per cent in weight. The gastric gland cells lost about 80 per cent in volume, and the nuclei 42 per cent; the intestinal epithelial cells 65 per cent, and the nuclei 53 per cent. Evidence of a decrease in the number of cells was not found. The histologic changes were less marked in young than in adult eels.

Liver.—Stephani (1923) found that, at necropsy, the liver in cases of acute nutritive disturbance (especially with signs of intoxication) almost always shows marked fatty changes, which are slight or absent in cases of chronic malnutrition. Stefko (1927a) observed a reappearance of blood-forming cells in the liver in famine-stricken adults as well as children. Some weights were recorded by Sedlezky (1924). Bean (1925, 1926) found an apparent depression of the weight of the liver associated with emaciation in 4,871 records of necropsies in hospitals of New Orleans and Baltimore. These records showed the liver averaging about 15 per cent heavier in the well nourished patients.

Junkersdorff (1921) found that the liver lost weight more rapidly than the body in dogs fasting eleven days. The fat of the liver either persisted longer than the ordinary fat or was replenished by immigration from the latter through the blood stream. Salvioli and Sacchetto (1921) noted a decrease in neutral fats and fatty acids, with the appearance of lipoids, in the liver cells of dogs and guinea-pigs starved for various periods, up to a loss of about 41 per cent in body weight. The distribution of fat in the liver cells varied in the two species. Wolff (1924) found that in lean white mice the slight amount of fat in the liver was removed within twenty-four hours of fasting, while in fat mice the larger amount of fat in the liver was not all removed in

three days. Geelmuyden (1923) reviewed the changes in content of glycogen and content of fat in the liver during fasting.

Mayer and his associates (1914) did not find any decrease in the mitochondrial granules of the hepatic cells in the rabbit or dog starved with or without water. Miller (1926), on the contrary, observed in underfed young rats a decrease in the size of the liver cells, and in the number of mitochondria, glycogen granules and cytoplasmic vacuoles. Münzer (1925) confirmed Arapow's discovery that the number of binucleated liver cells decreases in starvation, with variations according to the type of diet (sugar, fat, protein).

Trambusti (1896) noted in the amphibian, *Spelerpes fuscus*, a marked atrophy of the liver cells, with a slight decrease in the nuclei after it had been two and one-half months without food. Hartmann (1918), on the contrary, found in fasting tadpoles of the toad (*Bufo vulgaris*) an apparent increase in the size of the liver cells. Hertwig (1924) saw a marked decrease in the liver (he did not mention the cell size) in fasting tadpoles of *Rana fusca*. Berg (1924) found a large accumulation of fat droplets in the liver cells of the salamander during the winter period of fasting. This fat was evidently derived through the blood stream from the fat bodies, adipose tissue, muscle, skin, testis and other sources.

In the trout (*Salmo fontinalis*) fasting up to three months, Plehn (1914) noted a progressive disappearance of the fat in the liver cells, which was soon restored on refeeding. The glycogen appeared more resistant than the fat, and was never entirely exhausted. Cotronei (1922) ascribed the marked change in the structure of the liver in *Petromyzon* (in comparison with the larval *Ammocoetes*) to a condition of inanition during the period of metamorphosis. Esaki (1925) described atrophy of the hepatic cells and nuclear changes as taking place during starvation in the fish, *Oryzias latipes*. D'Ancona (1922, 1926, 1927b) made extensive observations on the liver of the eel (*Anguilla*) during a progressive inanition up to 657 days. The volume of the liver cell was reduced about 90 per cent, and that of the nucleus somewhat less. Hyperemia and relative fibrosis were noted.

Pancreas and Submaxillary Gland.—Aron and his associates (1925, 1926) found the decrease in the weight of the pancreas nearly proportional to that of the body in both acute and chronic nutritive disturbances in infants. There was a slight increase in the content of water of the pancreas. Stefko (1924), on the contrary, asserted that, in starvation, the pancreas does not lose weight as do most other organs, but often increases. In this connection, the weights recorded by Sedlezky may be consulted. The microscopic structure is unchanged in the earlier stages of inanition; but in later stages it shows marked hyperemia, hemorrhages, degeneration and necrosis (hemorrhagic pan-

creatitis). In chronic cases, there may be a hypertrophic cirrhosis. The islets of Langerhans soon disappear completely. Seyfarth (1920, 1924) and Jorns (1927) reached the opposite conclusion, finding an enlargement and an increased number of pancreatic islets in a case of death from starvation. Frequent transitions from acini to islets were found. Jorns described a slight atrophy of the pancreatic acini and an increase of the intralobular connective tissue.

Grinew (1912) reviewed the earlier literature on the controversy as to the possibility of the transformation of pancreatic islets into acini (and vice versa) through disturbances of nutrition. Cecil (1911) measured and counted the islets in six dogs. He concluded (confirming Bensley) that inanition does not have an appreciable effect on the number, size or structure of the pancreatic islets. Martius (1915) found numerous islets in the pancreas of a starved man, but did not succeed in producing an increased number of islets by the starvation of frogs, mice, guinea-pigs and chickens. Watrin (1924), however, asserted that, in rats starved six or seven days, in spite of pancreatic atrophy, the old islets proliferated and many new islets arose by transformation of the acini. Further data were cited by Jackson (1925). The question as to the effect of inanition on the pancreatic islets is apparently still unanswered.

The ratio of nucleus to plasma in the pancreatic cells of the white rat, normal and starved, was investigated by Dolley (1925). Ma (1924) found the mitochondria of the pancreatic acinus cells changed from filamentous to irregular granular form in guinea-pigs fasting up to twenty days. The normal structure was recovered on refeeding.

Submaxillary Gland.—Takagi (1925) observed little change in the ordinary alveolar cells of the submaxillary gland in young and adult cats fasting from twenty-four to ninety hours. The demilune cells, however, became larger, with more numerous secretory granules, but fewer mitochondria. The nuclei appeared darker and richer in chromatin.

Kidney.—A study of 4,871 records of necropsies performed in American hospitals indicated to Bean (1925, 1926) that the average weight of the kidney is about 15 per cent greater in well nourished than in emaciated persons. Emaciation in man apparently affects the kidneys less than the heart or the liver. Some weights bearing on this question were given by Sedlezky (1924). In a study of the white rat, Hayashi (1924b) placed the kidneys in the group of organs which lose weight more slowly than the body during inanition. Fronstein (1922) noted a characteristic diuresis and an increase of nonspecific infections of the urinary tract during famine in man. Schwarz (1927) similarly found a decreased resistance (as indicated by the cellular reactions) to experimental infections in the kidney in starving white mice. Wolff

(1924) noted in the cells of the renal tubules in fasting white mice a transient fatty infiltration which disappeared if the inanition was prolonged or if normal feeding was resumed.

Female Reproductive System.—The inhibition of menstruation by malnutrition that was noted by numerous observers in various countries during the famine consequent on the war, was found likewise by Anitschkoff and Sawodski (1922) in Russia. The effect may long persist in the form of a hypoplasia of the genital tract, which, according to Küstner (1926), increased in the Halle clinic from 0.8 per cent of the cases in 1919 to 1.5 per cent in 1925. Siemens (1926), however, doubted whether the decrease in the number of births in Germany during the war could be ascribed to inanition. The statistics do not show any significant change in the relative number of twins and triplets, in contrast with the observations of Richter (1926) on domestic animals.

In contrast with the relatively slight effect on the weight of the testis, Aron and his associates (1925, 1926) found a great loss in the weight of the ovary in dystrophic infants. Among 120 cases of starvation, in which the patients were aged from 7 to 40, Stefko (1924a, 1927, 1927a) could not find a single mature ovum. The follicles had undergone marked involution and atresia, and a few persistent primordial follicles were seen. The interstitial gland cells also had become rudimentary, and the ovary consisted chiefly of fibrous tissue. The age of puberty was greatly retarded. The persistent effects of earlier malnutrition were further shown by the examination in 1926 of 148 girls from 14 to 18 years of age. Of these, 28 per cent showed general hypoplasia of the sexual organs, 8 per cent infantilism and 21.3 per cent marked retardation of puberty (of menses, mammary development and the like). Some ovarian weights were noted by Sedlezky.

According to Richter (1926), a distinct reduction in fertility of domestic animals (sheep, goat, swine) was caused by underfeeding in Germany during the war. Especially in sheep there was not only a decrease in the number of births but a remarkable rarity of twin births. Blum (1924) found that a change of the sex ratio during underfeeding did not occur in white mice. Martino (1927) noted in starved or underfed hens ovarian disturbances and a suspension of ovulation, with recovery on proper refeeding. Arager (1925a) described an inhibition of gonadal development and a degeneration of the gonocytes in fasting tadpoles of the toad (*Bufo vulgaris*).

Male Reproductive System.—Aron, Lasch and Pogorschelsky (1925) and Aron and Pogorschelsky (1926) did not find any indication of a loss in weight or change in content of water of the testis in dystrophic infants. Anitschkoff and Sawodski (1922) ascribed the depression of sexual function in adult males during the Russian famine to atrophy of

the seminal vesicles and a decrease in the internal secretion. Stefko (1924a, 1924b, 1927, 1927a) made an extensive study of the question. Among 800 undernourished Russian boys between 7 and 16 years of age, cryptorchism occurred in 216 (27 per cent). The cryptorchism was ascribed to a secondary ascent of the testis, associated with a shortening of the cremaster muscle. The percentage of cryptorchism was somewhat less in Jewish (13 per cent) and Tartar boys (from 8 to 10 per cent), and occurred chiefly in boys between the ages of 10 and 13 years. Puberty was greatly retarded, and the external genitalia in general remained undeveloped. The testis in the starved boys presented a general atrophic destruction of the seminiferous tubules with repression of spermatogenesis and with proliferation of the connective tissue. Similarly, of thirty-five starved adult men (from 16 to 42 years of age), seventeen did not have spermatozoa in the seminiferous tubules. The Sertoli cells and the interstitial cells of Leydig appeared to be the most resistant to inanition. The persistent effects of earlier inanition were observed in the examination of 851 outpatient boys in 1926. Of these, 309 (36.7 per cent) showed symptoms of genital hypoplasia. Some weights for the testis were recorded by Sedlezky.

In adult mice starved from four to ten days, among which the maximal loss was about one third of the body weight, Stieve (1923) found little if any effect on the size of the testis or on spermatogenesis, but a marked atrophy of the interstitial cells. The penis, prostate and seminal vesicles appeared normal, and copulative ability was unimpaired. Saller (1926) similarly studied the effects of acute and chronic inanition with loss of about one fourth in the body weight in white mice. There was evidently a loss in the weight of the testis, and atrophy of both seminiferous and interstitial tissues, though individual variations made the conclusions somewhat uncertain. Stone (1924) found retardation in copulative age, spermatogenesis and development of the accessory sexual apparatus in rats underfed (at maintenance) for twenty days, the tests beginning at the age of 20 or 30 days; but he did not find any appreciable effect in tests beginning at 45 days of age.

In pigeons without food, according to Amantea and Martino (1925), sex desire is lost in a few days. Restoration of the sexual functions is much slower than recovery in weight on refeeding. This was observed also in chickens by Martino (1926, 1927). In cockerels, the effect of inanition on the germinative function of the testis (measured by breeding tests) appeared earlier and persisted longer after refeeding than that on the endocrine function. The endocrine function of the testis was measured by the size of the comb, which is markedly reduced by inanition.

For amphibians, the effects of inanition on the gonads was investigated by Arager (1925a) in tadpoles of the toad (*Bufo vulgaris*) and

by Champy (1922, 1924) in adult *Triton alpestris*. In the male triton, inanition inhibits spermatogenesis and causes an involution of the spermatogonia. It is held that the male sexual gland on refeeding may be transformed into the female.

Suprarenal Glands.—In dystrophic infants, the loss in weight of the suprarenal glands is nearly proportional to that of the body, according to Aron and his associates (1925, 1926). Stephani (1923) found that the fat content of the suprarenal cortex nearly or entirely disappears during acute infantile malnutrition (especially with intoxication), but is variable in chronic malnutrition. Fockermann (1925) and Stefko (1926b) described the changes in the suprarenal glands resulting from the Russian famine as occurring in three stages: (1) an acute stage, with occasional hemorrhages in the cortex, and oftener atrophy of the zona fasciculata and of the medulla, followed by proliferation of the reticular stroma, and by fibrosis in the medulla; (2) the appearance of degenerative changes in the zona glomerulosa, zona reticularis and especially in the medulla (with a fall in blood pressure), and (3) the final stage, with vacuolar degeneration of all cortical layers and complete destruction of the medulla; a change in the pigmentation of the skin does not occur. Stefko (1926) concluded that the third type of suprarenal involution with hypoplasia of the medulla may persist as a permanent condition, even after the period of inanition is past. The suprarenal weights in such cases (adult) appeared subnormal, averaging from 7 to 8 Gm. in males and 10 Gm. in females.

In fetuses from starved mothers, Stefko (1925a) found the suprarenal glands normal in appearance and structure. In the postnatal cases of starvation (1927a), in children, the suprarenal glands usually appeared above normal in weight. This increase was ascribed to general hyperemia (often hemorrhages) and also to an increase in connective tissue, especially in the medulla. The parenchymal cells of both cortex and medulla underwent progressive degenerative and atrophic changes, which were described in detail and which corresponded with the three stages described by Fockermann and Stefko. The observations by Stefko and other Russian investigators were also reviewed by Ichok (1925) and Newsoroff (1927). Some weights of the suprarenal glands were given by Sedlezky (1924).

In the white rat, Hayashi (1924b), as did most earlier investigators, found an increase rather than a decrease in the weight of the suprarenal gland during inanition. The cells showed a variable degree of shrinkage, somewhat proportional to the length of the test. Hett (1926, 1926a) studied the effect of acute or chronic inanition on the suprarenal glands of both younger and adult white and gray mice. Degeneration of the cortical cells to form syncytial masses, containing pigment occurred in the zona reticularis and adjacent zona fasciculata. The

effects were less marked in the medulla, where pigment granules were found in the capillary endothelium.

Thyroid Gland.—The importance of hypothyroidism resulting from the atrophy of the thyroid gland during famine has been emphasized by Curschmann (1921, 1923) and Lichtwitz. Fukushi (1924) found the thyroid gland in dystrophic infants subnormal in weight in thirty-four of forty-five cases. He summarized the changes as follows: surface, brownish red, frequently nodular; consistence, rather firm; size and weight, decreased; follicles, decreased; epithelium, cubical or often flattened; colloid, thin; interstitial connective tissue, somewhat frequently increased. Aron and Pogorschelsky (1926) found the weight of the thyroid gland (percentage of final body weight) above normal in dystrophic infants, and therefore concluded that it had lost little. Stefko (1925a) noted a normal appearance and structure of the thyroid gland in the fetuses from starved mothers; but (1927a) in malnourished children and adults, he observed that it was subnormal in weight, both absolutely and relatively. A microscopic examination revealed atrophy of the thyroid follicles, with a variable degree of epithelial flattening and desquamation, and also changes in the colloid content. Thyroid atrophy and involution during malnutrition were reviewed also by Ichok (1925) and Newsoroff (1927). Some weights of thyroid and parathyroid glands were given by Sedlezky (1924).

Mitochondrial changes in the thyroid epithelium were studied by Nicholson (1924) in rabbits and guinea-pigs subjected to various conditions, including a fast of from three to six days and a diet deficient in vitamin B. The filamentous mitochondria became fragmented and granular in structure. Chang (1925) confirmed Jackson's results showing atrophy or degeneration of the thyroid from chronic starvation in the albino rat.

Parathyroid Glands.—According to Curschmann (1923), the parathyroid glands may also be affected during inanition (Hungertetanie of Lichtwitz and others). The changes were described by Stefko (1927a), whose work was also reviewed by Newsoroff (1927). In malnourished children and adults, the parathyroid glands became considerably enlarged and hyperemic. Follicles appear containing a variable amount of colloid. The parenchyma is poor in cells (basophil and chief cells); sometimes mast cells are numerous. In some cases, the cells show hydropic degeneration.

Hypophysis.—Observations of the hypophysis, such as were made by Sedlezky (1924) in starved Russian children, were reported also by Stefko (1927a). Sedlezky did not find any change in the macroscopic appearance and but slight loss in the weight of the hypophysis. The anterior lobe (especially in children) appeared hyperemic, with a

variable degree of hypoplasia in the glandular tissue, a decrease in the number of eosinophil cells and a relative increase in the basophil cells and granules. There was also an increased formation of colloid. The changes, however, were of comparatively slight importance.

PARTIAL INANITION

The effects of partial inanition will be considered under the heads of deficiency of protein, deficiency of minerals, deficiency of water and deficiency of vitamins. The effects of a dietary deficiency of fat are ascribed chiefly to the associated deficiency in the fat-soluble vitamins, A, D and E. Burr (1928), however, found a caudal dystrophy in rats on diets fat-free (except for the known vitamins), and Baldwin (1928) described marked lesions in the testis and suprarenal glands in rats on a cholesterol-free diet.

EFFECTS OF A DEFICIENCY OF PROTEIN

Malnutritional Edema.—The generalized or localized edema frequently found accompanying chronic inanition in man ("famine edema") is probably associated with diets variously deficient in part, as well as with general caloric insufficiency. The available evidence, however, seems to indicate that a dietary deficiency of protein is the most important pathogenic factor. According to Lubarsch (1921), the characteristic lesions of the "Oedemkrankheit" include: (1) a marked depletion of fat and lipoids, with a persistence of lipoid remnants in muscle, cartilage cells and the suprarenal cortex, and increased lipoidal pigmentation in various regions; (2) atrophic changes with pigmentation, especially in the heart and the liver; (3) a variable destruction of red blood cells, associated with deposits of hemosiderin in various organs, and (4) a tendency to serous exudates and hemorrhages, especially in the alimentary canal, which are due to direct injury of the endothelial cells of the capillaries, and not to cardiac or renal dropsy.

Anitschkoff and Sawodski (1922) observed that in the Russian famine the sometimes enormous edemas were associated with a slow pulse rate and a weakened heart, without lesions of the kidneys. The location of the edema was variable, but oftenest in the face or the extremities. Beeuwkes (1926) described the Russian "hunger edema" in detail, and gave many photographs. The most characteristic symptoms were edema, polyuria without albumin, bradycardia and bulimia. The edema began first in the lower limbs, and extended variably upward. There was sometimes a general anasarca, amounting to 25 per cent of the body weight. The heart suffered severe damage, and sudden death from heart failure was common. The blood pressure was low, except in patients with arteriosclerosis. The gastro-intestinal symptoms were variable, but dysentery was characteristic. The nervous symptoms

were inconstant. In the blood, there was a tendency to hydremia. The red cell count sometimes decreased to 2,500,000, the average being 4,000,000. Leukopenia with a decrease in the count of polymorphonuclears and a relative lymphocytosis was frequent. Some authors maintained that the type of edema could be differentiated according to the leukocytic formula.

The factors in the pathogenesis of famine edema were discussed by Kusnezoff (1922), Kabanoff (1923) and N. Anitschkoff (1925). From his experience with "hunger edema" in Germany, Curschmann (1922, 1923) emphasized the atrophy of the thyroid gland as the most important lesion, the resultant edema being related to the myxedema of hypothyroidism. Jáureguy (1925) described an extreme edema of the legs, resembling elephantiasis, in an infant which had been fed for months on a thin vegetable soup. On suitable diet, the edema disappeared within a week. Hoelzel (1928), from experiments made on persons while fasting, concluded that protein starvation is the primary factor in the pathogenesis of nutritional edema. He found also that although colds were not ordinarily contracted during the prolonged fasting, they developed almost invariably after such periods, when edema was also prominent.

Experiments on Animals.—Stammers (1926) reported some hypertrophy of the suprarenal glands, disappearance of cortical lipoids and an increased epinephrine content in cattle with malnutritional edema (avitaminosis?). Moise and Smith (1924) and Smith and Moise (1924) found that in rats poisoned with chloroform regeneration of the hepatic cells occurred during fasting or on a diet of gelatin, though less rapidly than on diets containing gliadin or casein as the protein component. Thus, regeneration of the liver can occur on diets which do not permit general growth of the body.

Light (1927) observed that a deficiency of protein, phosphorus or vitamin A caused a lengthening of the estrus cycle in the albino rat. Hartwell (1925) noted a loss of fur in pied rats when potato or white bread was their sole source of dietary protein. The substitution of brown bread or oatmeal, or the addition of gluten, gelatin or caseinogen to the bread diet prevented the loss of fur.

Chang (1925, 1926a) tested Cramer's theory that tryptophan is a dietary essential as the mother substance of the thyroid hormone. He concluded that in white rats tryptophan is necessary for maintenance of the body, but probably not directly essential to thyroid activity. Abel and his associates (1925), from somewhat similar feeding experiments, reached the opposite conclusion that tryptophan is essential to the normal functioning of the thyroid gland, but held that most of the needed tryptophan can be supplied through the blood stream from other tissues of the body. The effects of a dietary deficiency of trypto-

phan on the thyroid gland are therefore comparatively slight, although general emaciation and death result.

According to Beard (1926), cystine cannot be successfully replaced by taurine, although both contain sulphur, in the diet of mice. Cystine is the limiting amino-acid factor in casein when the casein forms 12 per cent of the diet. The addition of 0.5 per cent of cystine causes growth at a rapid rate. The records of the experiments of Hayashi (1924, 1924d), including those on the effects of a deficiency of protein in the white rat, were inaccessible.

Although an increased amount of protein is required by the rat during reproduction and lactation (as was made clearly evident by Simmonds in 1924), Maynard and Bender (1928) found that in the rat during this period a 50 per cent ration of protein did not show any advantage over an 18 per cent ration.

EFFECTS OF A DEFICIENCY OF MINERALS

In this section of the paper will be included, first, some recorded observations on the effects of mixed or multiple deficiencies of minerals. The studies on rickets, although involving a disturbance in the relations of calcium and phosphorus, are considered under the head of vitamin D. McCollum, Simmonds and Becker (1925) found that the previously described "salt ophthalmia" in rats cannot be induced by feeding excessive amounts of any one element or ion. Possibly the effect of the salt factor is simply to damage the vitamin A present in the diet. Orr (1924) reviewed the significance of various mineral elements (calcium, potassium, iron, iodine and other elements) in animal nutrition and the effects of a deficiency of minerals in children and adults. The mineral balance is held to be even more important than the vitamins.

Haag and Palmer (1928) also emphasized the importance of the dietary balance of calcium, phosphorus and magnesium salts as essential for normal health and growth in rats. Various combinations resulted in an enlargement and a hemorrhagic condition of the suprarenals. One diet (with the content of calcium low and of magnesium and phosphorus (high) in a few cases apparently produced cystitis and phosphatic calculi. The salts also appeared to be important in relation to some of the vitamin requirements. Chidester, Eaton and Thompson (1928) believed that vitamins are effective through their mineral content.

Kligler and Geiger (1928) found that a dietary deficiency of any of the important salts (potassium, sodium, calcium), except magnesium, caused dwarfing of young rats. A deficiency in any of these salts also lowered the resistance to infection with trypanosomes.

Mori (1924) fed rabbits a diet of hydrous wool fat, bacon fat and cholesterol (a diet deficient therefore in protein and vitamins, as well as in salts) and produced degenerative changes in the teeth. Although

the enamel appeared normal, the dentinal ground substance was necrotic and the dentinal tubules filled with fat. The odontoblasts were atrophic, and fatty infiltration appeared in the pulp cells and connective tissue cells of the peridental membrane. Changes were also found in the cementum and alveolar bone.

Osborne and Mendel (1923) reported a failure of growth in rats when the salt content of the diet was reduced to 1 per cent or less. Complete recovery and growth were not obtained on the addition of salts to the diet later. The records of the work of Hayashi (1924, 1924d), including that on the effects of a deficiency of salts in rats, were inaccessible.

Yamasaki (1923) studied the effects of various dietary deficiencies on the gonads of white mice. The results of the deficiency of salts were similar to those of a deficiency of vitamins or of total inanition, including a disturbance of spermatogenesis in the testis and follicular atrophy in the ovary. This work was continued by Hirabayshi (1924), who investigated the effects of a deficiency of certain elements (sodium, chlorine, calcium, magnesium, potassium, phosphorus, iron), singly or in certain groups. Deficits of calcium, phosphorus and magnesium (individually) caused the most severe effects. Normal spermatogenesis apparently required the presence of various salts. The removal of one ion might result in the disturbance of other ions.

Thompson (1926) obtained normal growth in albino mice on a diet (skim milk powder 20, casein 24, starch 20, butter fat 32, yeast 2) with an ash content of only 0.7 per cent. A lowered hemoglobin content was noted, especially in the second generation. The bones were delicate. Obesity was common in old age, more frequently in the females. "Fatty infiltration was found in all the internal organs besides large quantities of fat in the usual storage spaces. There were a few cases of moist looking fur, but, more commonly, the fur was rather thin and without lustre."

In order to throw more light on the significance of mineral nutrition in animals, Hirsch (1925) reviewed the subject of minerals in plant nutrition. The cations K^+, Na^+, Ca^{++}, Mg^{++}, H^+ and Fe^{+++} and the anions Cl^-, NO_3^-, $SO_4^{=}$ and $HPO_4^{=}$ appear essential although the exact function of each is not clear. The question of mineral nutrition in plants was reviewed also by Palladin (1926), who compiled an extensive bibliography.

Deficiency of Calcium.—McCollum (1926) stated that "probably a lack of calcium, and the taking of a diet disproportionately rich in phosphorus, and lacking in vitamin D, are the faults of greatest significance in the diet of many Americans." This combination would (he thought) lead to rickets. This form of malnutrition will be discussed later under the head of vitamin D. The marked effect of slight variations in the

amount of dietary calcium was shown by Stepp (1925). Rats on a mixture of oatmeal, casein, dextrin and sodium chloride with 1.5 per cent calcium carbonate developed severe keratomalacia only; but with the calcium carbonate increased to 3 per cent, rickets appeared in addition to the keratomalacia.

Mellanby and his associates (1924) concluded that a shortage of calcium and calcifying vitamin is responsible for caries in the teeth of children. Toverud (1927) held that similar dietary deficiencies are the chief factor in producing dental caries of women during pregnancy. These conditions are probably closely related to rickets. Lebedev (1927) still adheres to the older view that rickets is not a specific disorder but a variable symptom-complex that may be produced by various means, including that of simple deficiency of calcium. Corlette (1928) held that a deficiency of calcium and phosphorus can cause a nervous disorder closely resembling that produced by a deficiency of vitamin B in man and lower animals. Among the morbid conditions involving these mineral deficiencies, in which nervous symptoms often occur, are polyphagia, earth-eating, bone-chewing, coprophagia, rickets, osteomalacia, spasticity, paraplegia, tetany, and the stijfziekte and lamziekte of South African cattle.

According to Simmonds (1924), the growth of rats on a diet in which the calcium content was low was only half the normal rate. Mother rats on such diets might produce and nurse young, but often they broke down on account of a loss of calcium from the skeleton. Kennedy (1926, 1926a) found that a deficiency of calcium in a diet otherwise adequate induced sterility in both male and female rats, probably through effects on the germ cells. The nature of the sterility was uncertain. Ovulation and the formation of normal corpora lutea took place, but signs of implantation or resorption did not appear in the uterus. Macomber (1927) concluded that the deficiency of calcium did not produce sterility in the adult rat, but did affect fertility by increasing the intra-uterine mortality. Since the prenatal skeleton is largely cartilaginous, the mother rat may be able to supply from her own reserves sufficient calcium for normal fetal growth. During lactation on the diet low in calcium, however, the maternal reserves are exhausted and the young soon become stunted in growth as a result of the deficiency of calcium.

Buckner, Martin and Peter (1925) found that larger eggs (yolks and white) were produced when calcium carbonate (crushed oyster-shell) was added to the corn-wheat-buttermilk diet of hens.

Hirsch (1925) and Day (1928) emphasized the indispensability of calcium for plant growth, as shown in beans, peas, buckwheat and other plants. The growth of the root is greatly injured by a deficiency of calcium, and a degeneration of the nuclei and the chlorophyl granules may result.

Deficiency of Phosphorus.—Stammers (1926) placed two adult albino rats on a diet without phosphates. They died in forty-one days with a loss of 40 per cent in weight. The yellow bone marrow was gelatinous, but the organs appeared normal. The suprarenal glands were not enlarged, and showed an abundant lipoid content in all three cortical zones. Light (1927) found that a deficiency of phosphorus in the diet prolonged the estrus cycle in the rat.

Eckles, Becker and Palmer (1926) made an extensive investigation of a disorder in cattle similar to that previously appearing in various parts of the world. The symptoms include depraved appetite ("pica") and markedly retarded growth and dwarfing of the adults with a disproportionate enlargement of their heads. The ovaries are atrophied, the estrum is inhibited, and fertility is markedly subnormal. The bones show osteomalacia and osteoporosis, especially in the ribs. The joints become exceedingly stiff and creak loudly. The disease is apparently caused by a deficiency of phosphorus (not calcium) and can be prevented or cured by feeding bone meal or simply inorganic phosphates.

Deficiency of Iodine.—The relationship between a deficiency of iodine and goiter is an unsettled question. McClendon and Hathaway (1924) published further data showing the relative content of iodine in the food and water from goitrous as compared with nongoitrous areas. Skinner (1924) presented twelve cases of congenital goiter ascribed to a deficiency of iodine in the maternal diet. The pathologic changes varied from a simple parenchymatous goiter without symptoms to marked enlargement with complete tracheal compression and dangerous cystic conditions. Marine (1924) gave an extensive review of the theory that simple goiter is caused by a deficiency of iodine. His conclusion was that "the gland cells begin to hypertrophy when the iodine store falls below a given level (less than 0.1 per cent in the animals studied), and continue this hypertrophy and hyperplasia until exhaustion atrophy or recovery supervenes. By anatomical recovery one means the involution of the active hyperplasia to the colloid or resting stage and not, as some have supposed, the disappearance of the thyroid enlargement." Tanabe (1925) similarly concluded from feeding experiments that the lack of iodine is the basic etiologic factor in the production of goiter (Struma diffusa parenchymatosa) in rats. Extremely small amounts of potassium iodide inhibited the glandular hyperplasia. The administration of iodine likewise prevents the condition of exophthalmia and thyroid hyperplasia in young trout (Hamre and Nichols, 1928), and inhibits the compensatory hypertrophy of the thyroid after partial resection in dogs (Schmitz-Moorman and Meis, 1928).

On the other hand, some are still skeptical regarding the theory that goiter is caused by a lack of iodine. Oswald (1927) of Zürich, for example, though admitting that in general iodine is valuable as a pre-

ventive and curative agent for goiter, denied that this proved deficiency of iodine to be the cause. Many persons in regions where there is supposedly a deficiency of iodine, do not have goiter, and many goiters occur in coastal regions, where there is an abundance of iodine. Liek (1927), who studied 1,286 cases in the Danzig district, for similar reasons opposed the theory that goiter is caused by a deficiency of iodine.

Deficiency of Iron.—McGowan (1924, 1924a) and McGowan and Crichton (1924) described a disorder in pigs the mothers of which had been fed an iron-poor diet of distillers' draff, maize and whitefish meal during pregnancy and lactation. The young pigs became fat and stocky in build and anemic (the hemoglobin content falling as low as 20 per cent), and showed enlarged hearts, effusions into the serous cavities, and sometimes, in the later stages, fatty changes and necrosis in the hepatic lobules. The disease appeared to be identical with the so-called cotton seed meal poisoning (really a disorder due to a deficiency in the diet, according to McGowan). It was found that the disease could be prevented and cured by the administration of ferric oxide in the food.

Hart and his associates (1925) observed that rabbits on a diet of cow's milk developed marked anemia. The addition of inorganic iron did not cure this anemia, unless there was also added fresh cabbage, alcoholic extract of cabbage, corn meal or chlorophyl. Recently, the same investigators (Waddell and others, 1928) showed that a similar anemia induced in young rats by a diet of milk was cured by the feeding of either dried liver (1.72 Gm. daily) or the ash of the same amount of liver (containing 0.5 mg. of iron), but not by the addition of the iron salts alone.

Deficiency of Other Minerals.—The effects of a deficiency of potassium on the development of the sea urchin (various species) was investigated in detail by Runnström (1925). There was a disturbance of the colloidal condition of the protoplasm, affecting lipoids and protein and distribution of the water. The mitotic figures were deformed in various ways. Amitosis sometimes occurred. The form of the segmented ovum was abnormal. The vegetative pole of the ovum was affected more than the animal pole. The gradient of sensitivity (from greater to lesser) was in the following order: mesoderm, entoderm, ventral ectoderm, dorsal ectoderm. Recovery was possible in normal sea water, if the injuries had not been too great.

That magnesium is necessary for the growth and maintenance of white mice was indicated by the experiments of Leroy (1926). Kligler and Geiger (1928), however, did not find any retardation in the growth of rats on diets deficient in magnesium.

McLean and Gilbert (1925) found that a deficiency of manganese caused chlorosis in spinach. The work of McHargue (1928) likewise

confirmed the theory that manganese is a nutrient necessary to plants. Warington (1926) found also that a deficiency of boron affected both the external morphology and the internal anatomy of the bean plant.

Hart, Steenbock, Waddell and Elvehjem (1928) concluded that copper in the diet was essential for a normal condition of the blood and the body. In anemic rats on a diet of milk, the improvement that follows the addition of dried liver or the ash of liver, corn or lettuce to the diet is ascribed to the presence of copper rather than of iron.[1] McHargue (1928) believed that copper is likewise necessary in the nutrition of plants.

Sievers (1928) found that a deficiency of nitrogen in the soil produces in arid regions a nutritional disorder of orchard trees with a peculiar effect on the development of the leaf and the structure.

The effects of a deficiency of oxygen observed by Vacek (1926) in white mice are of interest for comparison with the effects of deficiencies of minerals. There is a progressive hypertrophy of the heart (up to 30 per cent) and of the lungs. The increase in size of the heart is secondary to that of the lungs in an inverse fashion, the hypertrophy of the heart being greater in cases in which the lungs are insufficient in size.

EFFECTS OF A DEFICIENCY OF WATER

Since water forms a large and essential component of living protoplasm a deficiency of water (giving rise to aqueous inanition) in general has markedly injurious effects on all living organisms. Siebeck (1923) reviewed the general significance of water in the body, and the factors concerned in the production of edema. Staub (1924) traced the processes involved in the entrance of water through the intestinal wall, the exchange of water between the tissue cells and the blood or lymph and the excretion of water through the kidneys, lungs and skin. Staub held with Stolte (1923) that water is of fundamental importance, because protoplasm is in a colloidal state. Schiff (1924) and Thoenes (1924) likewise emphasized the importance of the water content of the body from the point of view of colloidal chemistry. Thoenes distinguished between free water, which is easily separated from the tissues, and bound water, which is colloidal. The bound water (in muscle) is normally about equal to the fat-free dry substance, but may become relatively reduced in certain abnormal conditions of exsiccation (toxic diarrhea).

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1. The factor of liver that is effective in anemia in rats is apparently different from that which is curative in pernicous anemia in man. The latter factor, according to Cohn and others (1928) is either a nitrogenous base or a polypeptide of which but 0.6 Gm. daily produces a pronounced response of reticulated blood cells.

The recent work on the effects of a deficiency of water may be classified as related to (*a*) effects on infants, (*b*) effects on the blood, (*c*) experimentally produced effects on animals and (*d*) effects on plants.

Effects on Infants.—Marriott (1923) stated that the two chief nutritional disorders of infancy are athrepsia (caused by quantitative or qualitative inanition) and toxicosis. The toxicosis or "alimentary intoxication" may arise in either athreptic or previously healthy infants. It is an acute disturbance marked by sudden loss of weight, exhaustion, fever, dry and grayish skin, usually diarrhea, vomiting and neural disturbances. The varied causes all result in depletion of the general water reserves. This depletion produces the marked fall in weight, and the changes in the blood (to be mentioned later).

Finkelstein (1921) held that among other effects a loss of water may cause infantile alimentary toxicosis. He believed, however, that the immediate pathogenic factor in this disease is a disturbance of the liver, which interferes with its detoxifying function. Stephani (1923) found fatty changes in the liver nearly constant in such cases. Stolte (1923) also recognized the loss of water as an important factor in the pathogenesis of infantile intoxication, but held that the dehydration affects the cell protoplasm throughout the body. Kleinschmidt (1923) thought the primary effect is on the intestinal wall, lesions of which permit the absorption of toxic materials (autotoxemia). Schiff (1924) and Schiff, Eliasberg and Bayer (1924) concluded that in human infants a shortage of water produces toxic symptoms only when the diet contains sufficient protein. Increased permeability of the intestine may permit the absorption of toxic factors (incompletely broken down proteins). These toxins, however, are usually detoxified in the liver, so that the hepatic lesions (often seen at autopsy) may be of primary importance. Further work on the relation of a deficiency of water to alimentary toxicosis will be cited later in the section on experimental results.

Rosenbaum (1924) gave a complete review of the literature together with observations on eighty-eight cases of infantile intoxication. He held that the clinical symptoms depend on the rapidity of the dehydration, which may be caused by diarrhea, vomiting or excessive pulmonary evaporation. In addition to the changes in the blood (to be mentioned later), an increased amount of liver fat was found, usually but not always. Fatty infiltration of the liver may occur also in nontoxic cases. The loss in skin turgor is characteristic. Definite changes in water content or the histologic structure of the brain were not observed (this is contrary to the observations by Bessau).

Schiff and Bayer (1925) observed that in children on a relatively dry diet (dry milk, potato flour, butter and sugar) the kidneys are

involved, resulting in a dehydration pyuria. Mild albuminuria, with variable casts, leukocytes and erythrocytes in the urine were noted. Renal abscesses may arise through bacteriemia, the resistance of the kidneys being reduced by the injurious effects of the dehydration.

Aron (1926) reviewed the various effects of a deficiency of water in children. Water is held necessary for the removal of waste products, but chiefly for regulation of the heat in the body. Of all the organs, the brain suffers first, which accounts for the cerebral symptoms that occur during a deficiency of water. The kidneys also suffer, resulting in oliguria or even anuria, urinary casts and leukocytes. Pyelitis is aggravated. Various other symptoms were discussed.

Effects on the Blood.—According to Marriott, the exhaustion of the water reserves of the body in infantile toxicosis is associated with marked anhydremia. The concentration of the blood is shown by refractometric determinations, and the reduction in total blood volume by Rowntree's method. The concentration and reduction of the blood cause circulatory disturbances, leading to decreased urinary secretion and disorders in various organs. Saxl and Donath (1924) concluded from experiments on rabbits that the reticulo-endothelial system plays an important rôle in regulating the water content of the blood.

Rosenbaum's observations and review of the literature confirmed, in general, the appearance of anhydremia, as indicated by the changes in water content, dry substances, serum albumin and viscosity, as well as in the erythrocyte count. In twenty-eight cases of infantile toxicosis, the increase in the erythrocyte count averaged 30 per cent, and ranged from 0 to 60 per cent. Some toxic cases did not show an increase, however, and some with (slowly rising) concentration of blood did not have any toxic symptoms.

In dogs subjected to thirst (method and details not given), Wettendorff (1899) found an increase in the number of red cells but a decrease in the number of leukocytes, hemoglobin content and density of the blood. In one dog subjected to "absolute inanition" for five days, the red cell count increased from 6,300,000 to 8,000,000.

Underhill and Roth (1922) observed that in rabbits complete deprivation of water led to an increase (up to about 25 per cent) in the concentration of the blood. After administration of water, the concentration of the blood rapidly fell to normal levels, as a rule, though in some cases death occurred in spite of the restoration of water. Keith (1924) produced a rapid dehydration of dogs without fatal results by the intravenous injection of saccharose or dextrose. From 7 to 10 per cent of the entire store of water was thereby excreted through diuresis within a few hours, without fatal results. The blood and circulation were rapidly restored to normal if an adequate amount of water was given within a few hours of the dehydration. Keith and Wheelan

(1926) obtained similar results, including a loss of weight of from 4.7 to 9.9 per cent in four dogs within twenty-four hours through diuresis. The blood was greatly concentrated, as shown by the measurements of the hemoglobin content.

In experimental intestinal obstruction in dogs, Foster and Hansler (1925) found that lack of intake of water together with vomiting caused a marked dehydration with increased viscosity of the blood, high concentration of red cells and of hemoglobin, a decreased volume of blood flowing in the extremities and a marked loss in weight. The blood pressure and the respiratory rate were each low, the pulse rate was almost doubled, and the rectal temperature was slightly subnormal. The skin became dry and inelastic, with marked shedding of hairs. The salivary secretion ceased, and the oral and nasal mucosae formed dry incrustations. Death was attributed to starvation.

Experimentally Produced Effects in Animals.—In addition to the work reported in the foregoing section on the changes in the blood, some further experiments with deficiency of water will be reviewed.

Engels (1904) showed by intravenous injections of physiologic sodium chloride solutions in dogs that the musculature, although forming only two fifths of the body by weight, absorbed about two thirds of the injected water. The skin absorbed about one sixth, the remaining sixth being distributed among the various organs. Skelton (1927), however, found that in cats, although the musculature absorbed the greater portion of the excess water when hypotonic solutions were used, it lost relatively little water when hypertonic solutions were injected. The skin, intestines and spleen, on the contrary, yielded water more readily during this dehydrating process.

Garofennu and Derevici (1924) studied the histologic changes in various organs of nine dogs on a dry diet for from four to nine days, with a maximal loss of 2,200 Gm. (the initial weight being from 13 to 19 Kg.). In the lungs, the alveoli appeared decreased in size, with some epithelial desquamation and extravasation of leukocytes and erythrocytes. In the kidneys, the glomerular capillaries were dilated, with cortical lesions in the advanced cases. The suprarenal cortical capillaries were less dilated than the medullary. The thyroid follicles were decreased and variable in size; the colloid and cytoplasm distinctly basophil. Granules of iron pigment appeared in the Kupffer cells of the liver; also in some other organs.

Some recent experiments on animals related to the aforementioned question of infantile toxicosis will now be reviewed. Various observers noted that hepatic lesions (fatty changes) usually result from a deficiency of water, and these lesions were held responsible for the toxic symptoms which follow (Finkelstein, Schiff and others). Schiff, Bayer and Choremis (1925) studied the associated depletion of glycogen in

the liver in puppies on a dry milk diet. When protein was added to this diet, both fatty changes and depletion of the glycogen of the liver resulted. But neither of these hepatic changes occurred in animals on the dry diet without protein. They therefore concluded that the changes in the liver are produced, not by the exsiccosis, but by the associated disturbances in intermediary protein metabolism. According to Schiff and Choremis (1926), disturbances in the carbohydrate metabolism are also evident under these conditions.

Kramár (1926) did not find symptoms of toxicosis in kittens on a diet of condensed milk, although there was a decrease in weight, a concentration of the blood serum, wrinkling of the skin and great weakness. In puppies, however, the symptoms were more pronounced and closely resembled infantile toxicosis with loss of weight, nervous phenomena (with final coma), vomiting and diarrhea, leukocytosis, renal symptoms (glycosuria, albuminuria, cylindruria), hyperglycemia, thickening of the blood, deep breathing, acidosis, and other familiar appearances.

Kramár (1927), using puppies with concentrated milk diets of varied protein content, concluded (as did Schiff and others) that dehydration does not produce toxic symptoms unless there is a large proportion of protein in the diet. The protein-rich diet also produced a greater degree of dehydration, in spite of the fact that it contained a higher percentage of water, than did the dry diet containing little protein. Protein is therefore of importance in producing the exsiccosis, as well as the subsequent toxic symptoms.

The pathologic changes produced by the dry diets (with high and with low content of protein) in puppies were described in detail by Kramár and Kovacs (1927). The liver was enlarged and light-brownish. Microscopically, there appeared marked hyperemia and fatty infiltration of the parenchymal cells. The fatty liver occurred in connection with a low content of protein as well as a high content of protein in the diets (which is contrary to the observations made by Schiff). The lesions of the kidneys were more irregular and inconstant, with both types of dry diets. Hyperemia was most pronounced among the straight tubules; while fat granules appeared chiefly in the convoluted tubules. Gastro-intestinal lesions (hyperemic or hemorrhagic spots in the mucosa) were inconstant, appearing only when the toxic condition was superimposed on intestinal disturbances. Ulcers rarely occurred. These lesions were found in connection with each type of dry diet, and were therefore produced by the exsiccosis without reference to the protein.

The aforementioned experiments all had to do with a deficiency of water in mammals. Some studies have also been made on amphibia. Durig (1902) noted that in frogs losing 32.6 per cent in body weight

by evaporation the average weight of the sciatic nerve decreased from 0.8439 Gm. (75 per cent being water content) to 0.6693 Gm. (51 per cent being water content).

Arager (1925) studied the development of frog tadpoles (*Rana temporaria; R. esculenta*) when the eggs were removed from water and placed in a moist chamber. Loss of water caused a marked reduction in the size of the body cavities, with displacement and deformity of the internal organs. However, the volumes of the organs and their histologic differentiation (studied especially in the eye) appeared nearly normal. Masses of mesenchymal tissue with normal nuclei formed thin strips between the organs.

De Almeida (1926) reviewed the literature on the effects of dehydration in amphibia, and reported original experiments. Rapid evaporation, he found, sometimes produced death in frogs that had suffered a loss of only 10 per cent in weight. The injurious effect he ascribed to the nervous system through abnormal cutaneous stimuli produced by the dessication. When the evaporation was slower, the water lost from the skin was replaced from the deeper tissues. Under these circumstances, serious symptoms did not arise until there had been a loss of from 20 to 30 per cent in weight. Death from dehydration of the inner organs sometimes did not occur until there had been a loss of 40 per cent in body weight.

Some experiments have also been made on earthworms. Schmidt (1918) observed that when earthworms (*Allolbophora fetida*) were dried slowly, they might survive a loss of 61.6 per cent in weight, or nearly 73 per cent of their water content. In most cases, the blood vessels in the dried skin became ruptured, which caused death. Adolph and Adolph (1925) studied the changes in the water content of the frog, flatworm and earthworm (*Lumbricus terrestris*) under various conditions. In the same species of earthworm, Jackson (1926) found survival possible only up to a maximal loss of 43 per cent in weight. Measurements of areas viewed in cross sections indicated that during exsiccation in the earlier stages the epidermis, body muscles, celomic cavity, intestinal wall and lumen had lost, roughly, in proportion to their size. But, in extreme exsiccation, a relatively larger proportion of the loss had been contributed by the celomic cavity and the intestinal lumen. The loss in the tissues appeared to be chiefly from the intercellular spaces, as the cells (except those in the epidermis) showed but slight changes in size and structure.

Effects in Plants.—The effects of a deficiency of water on growth and structure are especially evident in plants, as was shown by Folsom (1918) for various species of the crowfoot (*Ranunculus*). Some of the tests were carried to the second and third generations, but an

inheritance of the acquired characters was not observed. Fritsch (1922) and Fritsch and Haines (1923) similarly studied the changes produced by drought in various species of terrestrial algae. Rubner (1924) found that the growth of yeasts was markedly inhibited by dehydration in a 2 per cent solution of sodium chloride, although fermentation persisted up to concentrations beyond 4 per cent. The relations of dehydration to colloidal chemistry in growth and metabolism were discussed. A detailed review of the literature on the chemical and physiologic aspects was given by Palladin (1926).

(To be Continued)

Notes and News

University News, Promotions, Resignations and Appointments.—R. Kraus, director of the State Serotherapeutic Institute in Vienna, has been appointed director of Instituto Bacteriológico de Chile in Santiago.

Allen K. Krause, associate professor of medicine and director of Kenneth Dows Tuberculosis Research Laboratory of the Johns Hopkins University, has been selected as director of the Desert Sanitarium and Research Institute near Tucson, Ariz.

Emmerick von Haan of the University of Vienna has been appointed associate professor of pathology in the University of Arkansas, Little Rock.

In the school of medicine of the University of Louisville, Ky., George McLean Lawson has been appointed professor of bacteriology.

Charles L. Connor, formerly instructor in pathology in Harvard Medical School, is now associate professor of pathology and executive head of the department in the University of California Medical School.

Albert P. Krueger of the department of bacteriology and experimental pathology in Stanford University has been appointed associate in the division of general physiology of the Rockefeller Institute for Medical Research.

William B. Wherry, professor of bacteriology and hygiene in the University of Cincinnati, is spending one year as visiting professor in preventive medicine in the University of Philippines.

John A. Kolmer, Philadelphia, has been awarded the Mendel medal by Villa Nova College for his work on immunology.

At the University of Chicago, Harriet F. Holmes has been appointed research associate in the department of pathology, and Milton T. Hanke has been promoted to associate professor of biochemistry in the same department.

On the occasion of the dedication of a new laboratory for anatomy and physiologic chemistry the degree of doctor of science was conferred on Simon Flexner by the University of Pennsylvania, where Dr. Flexner was professor of pathology from 1899 to 1903.

Howard M. Jamieson, Harding, Mass., has been appointed pathologist to the Decatur and Macon County Hospital, Decatur, Ill.

J. Forest Huddleson, Michigan State College, East Lansing, has been directed by the U. S. Public Health Service to carry on field investigations of undulant fever in various parts of Europe and Northern Africa.

Clarence Cook Little, formerly president of the University of Michigan, is now director of the Jackson Laboratory for Cancer Research on Mount Desert Island, Me. The investigations are to be conducted from a broad, biologic point of view.

The Paris Academy of Medicine has awarded the Prince of Monaco prize of 100,000 francs to Professor Borel of Strasbourg for his work on the etiology of cancer.

United States Civil Service Examination for Bacteriologist.—An examination will be held to fill a vacancy in the United States Public Health Service, Honolulu. The entrance salary is $4,000 a year. The duties are bacteriologic investigations and research in the field of public health. Applicants will not be required to report for examination at any place, but will be rated on education, training, experience and fitness, and on publications or theses. Applications must be on file with the Civil Service Commission, Washington, not later than July 3 next.

American Association of Immunologists.—At its recent annual meeting the American Association of Immunologists elected officers as follows: president, Oswald T. Avery; secretary-treasurer, A. F. Coca, and councilor, S. Bayne-Jones.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

RENAL FUNCTION IN ARTERIAL HYPERTENSION. RALPH H. MAJOR, Am. J. M. Sc. **176**:637, 1928.

Observations indicate that many patients with a hypertensive condition, belonging to the group called "essential hypertension," do not, as commonly stated, have normal function of the kidney. They are unable to excrete normally two guanidine compounds: methylguanidine and methylguanidine acetic acid anhydride (creatinine).

PEARL ZEEK.

COMPOSITE CURVES OF CARDIOVASCULAR REACTIONS FOLLOWING ADRENALIN INJECTIONS IN TUBERCULOSIS PATIENTS. GEORGE E. DETRANA and JOHN G. HILLEBRAND, Am. Rev. Tuberc. **18**:626, 1928.

Composite blood pressure curves of patients with tuberculosis following the subcutaneous injection of 0.5 cc. of 1:1,000 epinephrine indicate: (1) Minimal, moderately advanced and greatly advanced cases have, in general, vagotonic reactions. (2) Recovery from the effect takes place within an hour in the minimal and moderately advanced cases, while the greatly advanced cases show a prolonged effect. (3) The pulse rate in the minimal case is affected least, that in the moderately advanced group most. The extremely advanced cases show a primary slowing of the rate (vagotonic). (4) The curves of the cardiac output are not characteristic. When arranged on the basis of the weight curves, patients that are gaining show a primary sympatheticotonic reaction, and the systolic blood pressure of those in the stationary and losing groups remains level or diminishes; the pulse rate of patients in the losing group shows the greatest increase.

H. J. CORPER.

MASSIVE COLLAPSE OF THE LUNG. L. SANTE, Ann. Surg. **88**:161, 1928.

The author believes that this condition results from the simultaneous inhibition of the cough reflex by toxic or reflex stimulus, and that this permits secretion to accumulate in the bronchi. Both factors must be present. The various theories, the clinical signs and the treatment are presented.

N. ENZER.

EDEMA AND REDUCTION IN EXCRETION OF WATER IN PERNICIOUS ANEMIA. E. MEULENGRACHT, POUL IVERSEN and F. NAKAZAWA, Arch. Int. Med. **42**:425, 1928.

Edema in pernicious anemia disappears during the remissions. Occult edema may be tested for by applying a tight rubber band about the extremity for two minutes. On removal, a positive test is indicated by a depressed groove in the skin. Accompanying the edema during relapses is a reduction of water excretion, as compared with normal, after giving a single large amount. The specific gravity of the hourly specimens of urine of the water test tends to remain fixed at a rather high level. Physicochemical examination of the blood in pernicious anemia reveals in many cases a decrease of the total protein of the plasma, with a proportional decrease of the colloid osmotic pressure. Some of the determinations of colloid osmotic pressure fell within the critical zone of from 270 to 240 mm. of water, as applied to the appearance of edema in cases of nephrosis. The decreased hemoglobin itself is not a factor in the abnormal water metabolism, as proved in eleven cases of posthemorrhagic anemia with no edema and normal excretion in the water test. Renal function seemed to be good in a series of thirty-four cases of pernicious anemia, as tested by the blood urea, thiosulphate test and by urinalysis for albumin and casts.

H. R. FISHBACK.

LORDOSIS AS A CAUSE OF POSTURAL ALBUMINURIA. M. LEWISON, E. B. FREILICH and O. B. RAGINS, Arch. Int. Med. **42**:440, 1928.

Of twenty-five children with exaggerated lordosis, three showed a persistent tendency toward orthostatic albuminuria, and three others showed the tendency inconstantly. Alkalinization of the urine had no particular influence on the secretion of albumin. The chemistry of the blood showed no essential deviation from normal. It is concluded that lordosis is not an important factor in the production of orthostatic albuminuria.

H. R. FISHBACK.

THE PARATHYROID GLANDS: THEIR RELATIONSHIP TO THE THYROID, WITH SPECIAL REFERENCE TO HYPERTHYROIDISM. E. P. MCCULLAGH, Arch. Int. Med. **42**:546, 1928.

A review is given of the functional relationship of the thyroid and parathyroid glands, especially under abnormal conditions.

More than 550 determinations have been made of serum calcium in patients with thyroid disease, before operation, after ligation of the arteries and after removal of the thyroid gland. In 5.1 per cent of all cases there was a hypercalcemia distributed evenly in hypothyroidism and in hyperthyroidism. Hypocalcemia occurred in 1 per cent of all cases, and in each instance it occurred in a patient convalescing from thyroidectomy. In 75 per cent of the cases in which comparison was made there was an average post-thyroidectomy drop of 1 mg. of serum calcium. This is considered due to operative trauma to the parathyroids, or to removal of parathyroid tissue.

H. R. FISHBACK.

THE PANCREATIC ACTIVITY IN DIABETES MELLITUS. S. OKADA, T. IMAZU, K. KURAMOCHI, K. HORIUCHI and T. TZUKAHARA, Arch. Int. Med. **42**: 560, 1928.

In a series of twenty-one cases of diabetes mellitus the external secretory efficiency of the pancreas was determined by the authors' new method. The disturbance of external secretion does not run parallel with the severity of diabetes. Amylolytic activity was reduced in one third of the cases, while proteolytic and lipolytic efficiency were decreased in over one-half. The activity of the pancreatic juice, as well as its volume, varies considerably. Nearly all old patients showed some enzyme deficiency.

H. R. FISHBACK.

COMPARATIVE EFFECTS OF IODIDE AND OTHER SALTS ON WEIGHT AND GROWTH OF THE BODY. P. J. HANZLIK, E. P. TALBOT and E. E. GIBSON, Arch. Int. Med. **42**:579, 1928.

Separate groups of rats were given complete and deficient diets, with the addition to the food of either sodium iodide, sodium sulphocyanate, sodium bromide, arsenic in solution of potassium arsenite, manganese sulphate, sodium borate or thallium acetate. The time periods extended from five to twenty-one months. The results are expressed in weight curves. The food consumption is also given. In both the adequate and the deficient dietary groups iodide gave some increase of weight and growth in the majority of animals. Reduction of weight and growth, or even death in some instances, was caused by the remaining salts given. Reduction in the amount of food consumed is believed to explain the weight loss in some groups.

H. R. FISHBACK.

EXPERIMENTAL OBSTRUCTIVE JAUNDICE. W. C. BUCHBINDER, Arch. Int. Med. **42**:743, 1928.

Jaundice was produced in puppies by ligation and division of the common bile ducts. The heart rate slowed rapidly in the first five days, and then more gradually for the next ten or fifteen days, after which there was an acceleration of

the rate. The heart rate of the puppies used as controls slowed as a result of age and growth until finally their rates averaged lower than those of the jaundiced puppies. Adult jaundiced dogs were more toxic than the puppies and exhibited an increase of heart rate. The initial bradycardia of jaundiced puppies may be vagal, the initial visceral sensory stimulus arising in the distended bile ducts. In decerebrate frogs sudden changes in pressure in the bile tracts cause a slowing of the heart rate. The central nervous depression occurring late does not play a part in slowing the pulse, since acceleration is the rule in the late periods. The inversion of the T wave in the electrocardiogram of jaundiced puppies may have a functional rather than an organic basis.

H. R. FISHBACK.

ROENTGEN-RAY THERAPY OF THE HYPOPHYSIS IN A PATIENT WITH ACROMEGALY. R. E. ALLEN and H. LISSER, Arch. Int. Med. **42**:703, 1928.

Roentgen therapy was administered to a patient with acromegalic bony deformities, adiposity, decreased sugar tolerance and headaches, polydipsia and polyuria. With antidiabetic dietary measures alone, symptoms improved, the patient lost weight and the blood sugar was kept within normal limits, although the sugar tolerance was not benefited. With roentgen treatment there was an improvement of sugar tolerance, and later a lowering of the slightly elevated basal metabolic rate.

H. R. FISHBACK.

SECRETIN NOT A HEMATOPOIETIC STIMULANT. J. T. KING, Arch. Int. Med. **42**:762, 1928.

Four rabbits given injections of secretin over a period of eight weeks showed no increase in red blood cells over four control rabbits. Secretin as ordinarily prepared contains, among other things, proteoses, peptones and histamine, any of which may cause a sudden transient rise of erythrocytes by concentration. Analysis of some data in the literature indicates the importance, in blood changes in rabbits, of considering the normal cycle of red blood cells of from around 4,000,000 to 7,000,000.

H. R. FISHBACK.

LACTOSE METABOLISM IN WOMEN. O. WATKINS, J. Biol. Chem. **80**:33, 1928.

During the last stages of pregnancy there is a more or less constant excretion of small amounts of lactose. During the last few days before delivery there is a sudden marked rise in the excretion of lactose, which reaches its height on the day of delivery. After delivery, the lactose excretion immediately drops to a low level where it remains for from two to five days. There is then a sudden and often tremendous excretion of lactose and, during the first few weeks of lactation, the fluctuations in the excretion of lactose are very marked. By the end of the first month after delivery the lactose excretion has assumed a constant and lower level, and this level slowly approaches the normal values for residual reduction of the urine as lactation progresses. The tolerance for lactose of most normal women is, in the intermenstrual period, the same as that of normal men, the tolerance dose being 10 Gm. Menstruation causes an increase in the tolerance of women for lactose, so that at this time many women show no urinary response to the ingestion of 20 Gm. of lactose. During pregnancy the tolerance for lactose is increased, being in some women three times as high as in other persons. Also the urinary response to the ingestion of lactose is slower in pregnant women, and the return to normal is correspondingly slow. During lactation the tolerance for lactose is apparently the same in every way as it is in normal men and in most women in the intermenstrual period.

AUTHOR'S SUMMARY.

SYNTHESIS OF VITAMIN B IN THE RUMEN OF THE COW. S. BECHDEL, H. HONEYWELL, R. DUTCHER and M. KNUTSEN, J. Biol. Chem. **80**:231, 1928.

Cattle, unlike the majority of the animal species, have the ability to grow to maturity, to produce normal offspring, and to produce milk of normal dietary

composition on a ration highly deficient in the vitamin B complex. The essential vitamin appears to be manufactured within the rumen as the result of the proliferation of a micro-organism of the genus *Flavobacterium*. Highly potent preparations of vitamin B can also be secured from this organism, in vitro, after culture on a synthetic medium.

ARTHUR LOCKE.

CUTANEOUS AND VENOUS BLOOD SUGAR CURVES IN NORMAL INDIVIDUALS AFTER INSULIN AND IN LIVER DISEASE. M. FRIEDENSON, M. ROSENBAUM, E. THALHEIMER and J. PETERS, J. Biol. Chem. **80**:269, 1928.

The influence of the administration of carbohydrates and of insulin on the relative concentrations of dextrose in the arterial and the venous blood is discussed.

ARTHUR LOCKE.

MYXEDEMA FOLLOWING TREATED AND UNTREATED THYROTOXICOSIS. W. O. THOMPSON and P. K. THOMPSON, J. Clin. Investigation **6**:347, 1928.

An analysis has been made of the cases of thyroid intoxication associated with myxedema from the Thyroid Clinic of the Massachusetts General Hospital. Of the cases in which treatment was given, myxedema developed in less than 1 per cent following operation, and in about 4 per cent following roentgen therapy. With roentgen treatment myxedema developed as late as five years after treatment. Myxedema may also occur without any treatment. Many patients show low metabolic rates without myxedema. Thyroid therapy in cases of myxedema should be stopped for test periods, to prove whether or not the condition is permanent.

H. R. FISHBACK.

THE VOLUME OF URINE AND THE RATE OF EXCRETION OF UREA BY NORMAL ADULTS. E. MÖLLER, J. F. MCINTOSH, D. D. VAN SLYKE and E. M. MACKAY, J. Clin. Investigation **6**:427, 467, 485 and 505, 1928.

Blood and urine urea values and the volume of urine excreted hourly in six normal adults furnished data for determining the rate of the excretion of urea in relation to its concentration in the blood. Below a certain minute volume of urine, about 2 cc. the "augmentation limit," the excretion of urea falls at a rate proportional to the square root of the volume. About the augmentation limit, the excretion of urea is directly proportional to its concentration in the blood, and its minute-output represents the urea content of a maximum blood volume. This blood volume is termed the "maximum clearance," with an average value of about 75 cc. The "standard clearance" is the blood volume containing the excretion of urea for one minute, with the volume of urine for one minute at 1 cc. The mean value is 54 cc.

The urea values have been determined in normal children and compared with those for adults. The ratios for size are expressed in terms of body surface. The mean surface area of adults, aged 25, is taken as 1.73 square meters. Excretion of urea is found to vary directly as the surface area. When thus corrected, data for children give the same normal values as those for adults for the augmentation limit, and for maximum and standard clearances. In persons, between 62 and 71 inches (1.57—1.80 meters) in height, the correction for body size does not exceed 5 per cent in the determination of standard clearance.

The urea values have been determined in a number of patients with nephritis. The curves showing the excretion of urea are generally found to be lower than normal curves. This factor, as well as the blood urea value, must be considered in determining the extent of damage in a diseased kidney. The loss of renal function may exceed 60 per cent before the blood urea reaches even a high normal level. The standard clearance and maximum clearance were equally sensitive as indicators of damaged renal function in patients with nephritis.

The urea values were determined in normal persons and patients with nephritis at intervals throughout the day. The normal persons show a sharp drop in the

standard clearance after rising. There is a gradual rise during the late morning, followed by a drop during the early afternoon, and a second rise during the late afternoon and evening. The patients with nephritis give marked and irregular variation of the standard clearance curves. The period of least variation of values is during the forenoon; the taking of breastfast has no effect.

H. R. Fishback.

The Mechanism of the Inflammatory Process (Electrophoretic Studies). H. A. Abramson, J. General Physiol. **11**:743, 1928.

Quartz particles and certain other inert particles were found to move cataphoretically in certain soft gelatin gels with the same velocity as in the sol. The speed is a function of the true viscosity of the sol or of the gel, and the presence of gel structure does not affect it in the soft gels. It is proportional to the applied difference of potential. While in certain sols gelatin results in increased stiffness, with increase of apparent viscosity, this is not accompanied by a change in true viscosity, as measured by the application of high pressures, and it does not affect the migration rate. Red cells in these gels showed a marked difference in behavior; their migration rate is about twice that of the quartz particles, and their absolute velocity is slightly decreased by the presence of the gel. In stiffer or more concentrated gels, red cells, leukocytes and quartz particles all move with the same initial velocity. If mechanical softening of these gels is induced, the red cells presently assume their more rapid rate of movement. Since it has been previously shown that injured connective tissue is probably electropositive to the blood stream, and since the cataphoretic velocity of the negatively charged leukocytes in serum and plasma is about 0.5 microns per second per volt per centimeter, there is a force which may explain leukocytic emigration through the gel structure of the capillary wall.

H. E. Eggers.

High Blood Urea, Non-Protein Nitrogen, Creatinine and Uric Acid Values in a Case of Brain Tumor. H. A. Freund, Warthin Ann. Vol., 1927, p. 691.

The presence of large amounts of nonprotein nitrogen and urea in the blood has been associated with advanced nephritis. In the case reported by Freund, a physician, aged 53, showed blood urea values varying from 247 to 402 mg. per hundred cubic centimeters during a period of two weeks. The maximum blood uric acid value recorded was 21 mg. per hundred cubic centimeters, while the highest blood creatinine value was 11 mg. per hundred cubic centimeters. The urinary observations were not of the character of those found in acute nephritis. During the following month, all of the nitrogenous values gradually dropped to normal and remained so until the patient's death, three months after the repeated high nitrogenous values were recorded.

Autopsy revealed a cerebral fibrosarcoma. The kidneys showed no significant pathologic changes. The clinical sequence of events was entirely consistent with the symptomatology of a slowly infiltrating tumor of the brain.

Walter M. Simpson.

The Function of the Blood Liquor Barrier. V. Kafka, Deutsche Ztschr. f. Nervenh. **105**:50, 1928.

Kafka again writes of the barrier between blood and spinal fluid, contending that its anatomic site is not in the meninges or cortical blood vessels, but in the choroid plexus alone. He denies the theory that fluid is formed by simple dialysis. The permeability of the barrier is discussed in general, but the author admits that although its function is of greatest importance in the etiology and therapy of nervous and mental diseases, the exact function is unknown.

Roy Grinker.

PHOSPHORUS POISONING AND THE ACTION OF INSULIN IN ANIMALS. H. J. ARNDT and E. GREILING, Virchows Arch. f. path. Anat. **67**:243, 1928.

In rabbits, neither insulin nor sugar alone prevented the characteristic changes of phosphorus poisoning: fatty degeneration of the liver and loss of glycogen from the liver and muscles. With insulin and sugar together, fatty degeneration was not observed after phosphorus poisoning, and formation of glycogen took place in the liver; however, death of the animals was not prevented. Hence, the power of the liver to form glycogen, though reduced, is not entirely lost in phosphorus poisoning. The formation of glycogen in the renal epithelium did not occur, with or without insulin. The heart muscle appeared to be protected from loss of glycogen more than the skeletal muscle. No change in blood fat or cholesterol was observed; there were only inconstant changes in the lipoids of the suprarenal glands.

B. R. LOVETT.

EFFECT OF EXCISION OF THE ADRENAL GLANDS ON THE OVARIES IN MICE. KIYOSHI MASUI, Endokrinologie **2**:49, 1928.

In ninety-three mice the adrenals were removed, in seventy-one totally. The animals survived the operation on the average of six days. The mice that lived longer than twelve days regained their weight promptly, and all symptoms following the removal of the adrenals disappeared again.

The mortality rate was 73.3 per cent in the animals operated on previous to the sexual maturity, but only 52.3 in those after maturity. Mice in which regenerated adrenal tissue was found did not show symptoms, or only for a short time.

In some of the animals on which operations were performed accessory adrenals were found, which consisted only of cortical substance. Those animals did not show any nutritional disturbance.

The complete removal of the adrenal glands is followed in mice by a marked atrophy of the ovaries. The ova are degenerated, the interstitial tissue is hyperplastic and the formation of fresh corpora lutea ceases. A secondary atrophy of the endometrium of the uterus is observed.

These changes can be recognized seven days after operation and are maximal after from ten to fourteen days. Later, the ovary returns to normal. Masui believes that the atrophy of the ovary is caused by lack of the internal secretion of the adrenal glands, not by nutritional disturbances.

C. A. HELLWIG.

Pathologic Anatomy

EPIDERMOID CARCINOMA OF THE CERVIX UTERI. KARL H. MARTZLOFF, Am. J. Obst. & Gynec. **16**:578, 1928.

Martzloff's study is based on seventy specimens of cancer of the cervix in which biopsy material was available for a later comparison with the remainder of the uterus. The author found that in one third of the cases studied a study of the biopsy material in carcinoma of the cervix failed to show the predominant type of cancer cell in the remainder of the organ. Therefore, studies of biopsy material merely for a determination of prognosis to the patient are more or less inaccurate.

T. J. KOBAK.

THE DIAGNOSIS OF ENDEMIC YELLOW FEVER. W. H. HOFFMANN, Am. J. Trop. Med. **8**:563, 1928.

From our considerations the conclusion is drawn that the diagnosis of endemic yellow fever is not as easy as that of the severe epidemic form though the former is even more important as shown by the survey and control operations, which in the endemic centers always must be preceded by exact diagnostic work if they are to be effective, and an opinion on definite results of the hygienic measures can be formed only if the diagnostic service is based on completely reliable methods.

With great care and experience on the part of the responsible authorities it will often, in spite of all difficulties, be possible to make the clinical diagnosis in cases in which yellow fever is suspected if each case is especially examined for this condition until its nature is clear.

As long as clinical methods are insufficient and bacteriologic methods do not exist, the anatomic diagnosis is decisive and should be made in all fatal cases in the endemic territory in which death is due to an infection suggestive of yellow fever, or in mild cases in the monkeys infected with the blood of patients suspected of having yellow fever. This is probably the quickest and most reliable way to prove the presence of endemic yellow fever in a doubtful area.

AUTHOR'S SUMMARY.

THE FATE OF THE TUBERCULOUS CAVITY. FELIX BAUM, SOL MEBEL and ALLEN KANE, Am. Rev. Tuberc. **18**:596, 1928.

The allergic stage in which tuberculous cavities are formed may be determined by the shape of the cavities and by the thickness of their walls. The fate of the cavity depends on the stage of its formation. There may be two types of cavities formed in the second stage: one through caseation, called "sequestrum cavity," and the other formed through liquefaction-necrosis, called "concentric." The kind of tuberculosis resulting in liquefaction-necrosis is more readily absorbed. There is a difference between the healing and the repair of a cavity. The latter process occurs in the third stage, in which the cavity is collapsed and masked by a thickened pleura. Healing takes place in the first two stages by connective tissue replacing the destroyed parenchyma. It is believed possible that healing can occur through the regeneration of parenchyma. Infraclavicular cavities, because of their location and the stage in which they are usually formed, show a strong tendency to spontaneous healing.

H. J. CORPER.

TUBERCULOUS ENTERITIS. BENJAMIN GOLDBERG, HENRY C. SWEANY and ROBERT W. BROWN, Am. Rev. Tuberc. **18**:744, 1928.

The postmortem observations of gastro-intestinal changes in 230 patients who died from pulmonary tuberculosis are reported. Tuberculosis of the gastro-intestinal tract should be considered with relation to the lymphatic system. The early lesion may be different depending on the age, race and immunity of the host with a tendency to be more exudative in the young and nonimmune person. Although in a minority of cases the lesions are definitely exudative, showing only polymorphonuclears, lymphocytes and epithelioid cell infiltration, with no giant cells and few tubercles, most cases show a mixture of exudative and proliferative processes, so that a definite classification cannot be made on such a basis. Incipient lesions are found most frequently, first, in the lymphoid tissue above the ileocecal valve; secondly, in the cecum at the point where the food-current strikes the cecum; thirdly, on the margin of the ileocecal valve, and very rarely at isolated points in the ileum or colon. Concomitant diseases are not uncommon, some of which are related to the tuberculosis while others are not. Acute appendiceal lesions are important and should be studied carefully with the view of removal before perforation and fecal abscesses result.

H. J. CORPER.

THE ASSOCIATION OF TUBERCULOUS LYMPHADENITIS AND GENITO-URINARY TUBERCULOSIS. F. J. LUSSMANN, Am. Rev. Tuberc. **19**:95, 1929.

The practical conclusion is deduced that if in patients with pulmonary tuberculosis, especially in advanced stages, a tuberculous involvement of the external jugular vein group of lymph nodes or of the intercostal lymph nodes is found, special attention should be paid to the genito-urinary system, as it is probably already tuberculous or in danger of becoming so. From a theoretical and immunologic standpoint this offers also a demonstration of the interesting fact

of simultaneous or rather constantly successive tuberculous disease of two physiologically different organs, which possess, however, the embryonic relationship of descending from the same primary germ layer, the mesoderm. These two groups of organs show a more constant simultaneous involvement by tuberculosis than does any other group of organs except that of the intestines and larynx.

H. J. CORPER.

THE SCAPULAE OF THE CHINESE. H. D. KERR, Arch. Int. Med. **42**:508, 1928.

Various observers have reported on the relative frequency of different scapular types. The scaphoid type shows a decreasing frequency in the older age periods. Either the scaphoid scapula is converted to another form with increasing age, or the scaphoid type is eliminated by death. Observations were made on 546 Chinese from 15 to 78 years of age. The scaphoid scapular type gradually decreased from 82.1 per cent at from 15 to 20 years of age to 15.4 per cent at the age of 60 or more, which is interpreted as indicating an increased mortality among persons with scaphoid scapulae. No relation was found between the type of scapula and the occurrence of syphilis or tuberculosis.

H. R. FISHBACK.

EXPERIMENTAL ULCERATION OF THE ESOPHAGUS. J. FRIEDENWALD, M. FELDMAN and W. F. ZIRN, Arch. Int. Med. **42**:521, 1928.

Traumatic ulcers were produced in dogs by removing portions of the wall of the esophagus through an esophagoscope. Both superficial and deep ulcers were rendered chronic by the administration of 7.5 cc. of 10 per cent hydrochloric acid through a tube four times daily. This could be continued for several months. Roentgen examination demonstrated defects in outline at the site of ulceration, or spasm. Large penetrating ulcers sometimes simulated diverticula. By esophagoscopic examination the ulcers could be studied directly. They frequently perforated because of the thinness of the wall in dogs. The uncomplicated ulcers healed quickly without distortion of the wall, and without the fixation which may occur in human beings.

H. R. FISHBACK.

XANTHOMATOSIS AND THE RETICULO-ENDOTHELIAL SYSTEM. R. S. ROWLAND, Arch. Int. Med. **42**:611, 1928.

Xanthomatosis is a disorder of lipoid metabolism manifested by the storage of lipoidal substances in the reticulo-endothelial system, and further, by a hyperplasia of that system. There may be associated destructive processes in the membranous bones, exophthalmos and diabetes insipidus (Christian's syndrome). The two cases presented, and twelve others collected from the literature, show a generalized visceral xanthomatosis of this type. A review of the literature indicates the manifold systemic disturbances that may occur with lipoid imbalance, or lipoid gout, namely, Niemann's and Gaucher's diseases; dwarfism, infantilism, dystrophia adiposogenitalis, diabetes insipidus or mellitus, and other disorders of the ductless glands; certain diseases of the kidney; some disorders of the liver, and various obscure tumors.

H. R. FISHBACK.

GENERALIZED GRANULOMATOUS LYMPHADENITIS ASSOCIATED WITH DIFFUSE PROGRESSIVE FIBROSIS OF THE LUNGS. C. L. CONNOR, Arch. Int. Med. **42**:822, 1928.

A case is presented of a boy with general enlargement of lymph nodes, and progressive consolidation of the lungs, who died in thirty days. Tissues taken at autopsy showed progressive fibrosis of the lungs, moderately hyperplastic bone marrow, and in places in the lymph nodes a mixed cell picture resembling Hodgkin's disease.

H. R. FISHBACK.

AGRANULOCYTOSIS (SCHULTZ) AND THE AGRANULOCYTIC SYMPTOM COMPLEX. W. C. HUEPER, Arch. Int. Med. **42**:893, 1928.

The given description of agranulocytosis is from five observed cases, and from reports in the literature. Clinical features are: sudden onset in a healthy person, or more insidious onset after a period of ill health; high continued fever with chills, high rate pulse of poor quality, sore throat with ulcers of the throat and mouth, and enlarged cervical lymph nodes, jaundice, diarrhea, indefinite bacterial indications in the throat and blood, and marked decrease of leukocytes, especially the granulocytes. Pathologically, the ulcerations of the mouth vary from superficial erosions to deep gangrenous processes involving the larynx and esophagus. Ulceration may be found elsewhere in the gastro-intestinal tract, and on the vulva, vagina and cervix. The lungs frequently have scattered small areas of consolidation. There is cloudy swelling. The lymph nodes and spleen are hyperplastic. Red marrow is present in the long bones. Various diseases with secondary agranulocytosis may resemble essential agranulocytosis (Schultz).

H. R. FISHBACK.

TABES DORSALIS: PATHOLOGY AND PATHOGENESIS. GEORGE B. HASSIN, Arch. Neurol. & Psychiat. **21**:311, 1929.

The histologic changes in tabes dorsalis may be classified as degenerative and inflammatory. The degenerative changes involving the posterior columns and the arachnoid portion of the posterior roots are a primary process due to disturbed circulation of the tissue fluids in the spinal cord, especially in its posterior columns. The inflammatory changes occur in the dura and the pia-arachnoid, in which they provoke reactive phenomena. In the dura they occur as infiltrations with hematogenous elements and vascular changes typical of syphilis; in the arachnoid they appear as proliferation of the cells of the arachnoid (arachnoid or mesothelial cells). These are most likely what Richter designated as "granulation" cells. The arachnoid cells invade the perineural spaces, obstruct them, and thus interfere with the flow of cerebrospinal fluid from the subarachnoid space, indirectly causing phenomena of stasis in the spinal cord. The stasis results in rarefaction of spinal cord tissue and its ultimate sclerosis, which shows as islands of degeneration in the posterior columns. The pia-arachnoid phenomena are secondary to the distinctly inflammatory conditions of the dura and the epidural space; invading the subjacent structures, they result in the changes just mentioned. The strangulation phenomena of Obersteiner and Redlich, Nageotte, Richter and others are contributory but are not the main factors. Many clinical phenomena can be better understood on the basis of the epidural origin of tabes with involvement of the peripheral nerves in the epidural space. Future studies of tabes should be centered on the epidural space, including both the spinal and the periosteal dura, as well as on the spinal nerves before they leave the intervertebral foramens.

AUTHORS' SUMMARY.

CHONDROMYXOSARCOMA OF THE SIXTH CERVICAL VERTEBRA. EDWARD A. SHARP and WILLIAM F. JACOBS, Arch. Neurol. & Psychiat. **21**:381, 1929.

A vertebral tumor of the type of a chondromyxosarcoma of the sixth cervical vertebra and intervertebral disk, which produced compression paraplegia with the clinical symptoms, at the onset, of amyotrophic lateral sclerosis was presented. The spinal cord showed marked flattening from pressure outside the dura but not any degeneration of any portions of the structure of the cord. Multiple metastases were present in the lungs which showed tumor masses of the same character as that found in the vertebra and spinal canal.

AUTHORS' SUMMARY.

CHRONIC ULCERATIVE COLITIS ASSOCIATED WITH MALIGNANT DISEASE. J. A. BARGEN, Arch. Surg. **17**:561, 1928.

From 1916 to 1927, twenty-three cases of chronic ulcerative colitis with symptoms of malignancy occurred at the Mayo Clinic. In twelve of the cases, there

were varying degrees of obstruction. Roentgen examination demonstrated filling defects in eight. In fourteen of seventeen cases the malignant condition was carcinoma, six of which were multiple; in two, lymphosarcomas, and one, lymphatic leukemia. Bargen believes that the development of polyposis and chronic ulcerative colitis increases the chances of malignancy.

N. ENZER.

BLOOD SUPPLY TO THE APPENDIX. H. KOSTER and M. WEINTROB, Arch. Surg. **17**:577, 1928.

Gross' method of vascular injection was applied to 100 normal and pathologic appendixes. There was demonstrated seven primary branches of the appendicular artery, six of which divide into two encircling branches. These in turn divide into a smaller superficial layer and a larger deep layer. These layers anastomose each other. Anastomoses can be demonstrated in the meso-appendix.

N. ENZER.

THE EFFECT OF LIGHT ON BLOOD AND TISSUE CELLS. W. R. EARLE, J. Exper. Med. **48**:667 and 683, 1928.

Observations were made on hanging drops of blood obtained from veins in the ears of rabbits and exposed to irradiation. It was found that the red cells began to swell after from fifteen to thirty minutes, and then lost their hemoglobin, sometimes gradually, sometimes with explosive rapidity, hemolysis being complete at the end of from 90 to 110 minutes. Some cells also showed coagulation. In blood diluted with Locke's solution, the cells became hemolyzed one at a time instead of all at once. Hemolysis occurred at the same rate with white light and with light from each of the following spectral zones: (*a*) from 430 to 550 microns, infra-red; (*b*) from 475 to 630 and 690 microns, infra-red, and (*c*) 600 microns, infra-red. Washed cells underwent the same process of degeneration. The presence of air was apparently necessary, since in flat preparations from which air was excluded, degeneration did not take place. Changes in the white cells became evident only when hemolysis of the erythrocytes was complete, suggesting that substances liberated from the latter might be responsible for the degeneration of the former.

In the presence of autogenous red cells, fibroblasts from the hearts of embryo chicks also underwent degeneration when exposed to light, in the course of from three to four hours. The cells showed first an increase in the refractive index, followed by massive formation of vacuoles and sometimes by coagulation. The same wave length zones were active as in the case of the red cells, but the rate of degeneration varied markedly with light from the different zones, being slower through the blue or red filters and more rapid through the green filter. The presence of red cells was apparently necessary for the reaction, as in their absence only slight degeneration could be found even after from twelve to twenty-four hours.

B. R. LOVETT.

PRIMARY CARCINOMA OF THE FALLOPIAN TUBES ASSOCIATED WITH TUBERCULOSIS. WILLIAM P. CALLAHAN, FRANCES H. SCHILTZ and C. ALEXANDER HELLWIG, Surg. Gynec. Obst. **48**:14, 1929.

Primary carcinoma of the fallopian tubes has been reported in 196 cases. Tuberculosis occurs in 1 per cent of all gynecologic cases, but the combination of primary carcinoma of the tubes associated with tuberculosis of the tubes has been reported only six times, the authors' case making the seventh. Secondary carcinoma of the tubes associated with tuberculosis is also extremely rare. The pathologic diagnosis of primary carcinoma of the tubes associated with tuberculosis must not be confused with the atypical carcinoma-like proliferation which is so common in tuberculous salpingitis. Extreme care must be exercised in making a pathologic diagnosis, so as not to confuse some of the inflammatory processes occurring in carcinoma of the tubes with tuberculosis. The consensus

of opinion regarding the etiology of these conditions is that the one is an accidental complication of the other, and although the tuberculous process is usually the older, it can not be proved that it is the cause of the carcinoma. One case was reported of a primary carcinoma of the right tube and tuberculosis of the left tube. The prognosis is unfavorable. Early radical operation is the only treatment which offers any success. After two years the authors' patient showed no metastases and was in good health, with the exception of the presence of a fistula.

AUTHORS' SUMMARY.

INHERITED EPITHELIAL DEFECTS IN CATTLE. F. B. HADLEY and L. J. COLE, Research Bulletin 86, Agricultural Experiment Station of University of Wisconsin, 1928.

New-born calves exhibiting characteristic lesions of the skin and the mucous membrane were reported from a number of herds of Holstein-Friesian cattle in Wisconsin. The following lesions were characteristic: defective formation of the skin below the knees; one or more undeveloped claws; deformed ears, due to rolling of the margins and growing together of the surfaces brought into contact, indicating that the lesions antedated birth by some weeks; defects in the integument of the muzzle and in the mucous membrane of the nostrils, tongue, hard palate and cheeks. The defect does not appear to be associated in any way with faulty nutrition or with thyroid or other disturbances of the internal glands of the mother. Neither is it due to specific infection. The defect appears to be specific, and is designated epitheliogenesis imperfecta neonatorum bovis. The affected calves are carried to full term and are of normal size at birth. Some appear strong when born, others are weak and unable to stand, and all soon become debilitated and die even when given appropriate treatment and good care. Death is apparently attributable to a septicemia (blood poisoning) which develops as a result of infection gaining entrance to the body through the raw surfaces of the lesions. Studies of the microscopic changes revealed marked pathologic changes. Whether the epithelial structures had reached full development and then retrogressed, or whether the condition was due to incomplete or arrested development, is an open question. The latter conclusion appears the more probable. The method of inheritance of the epithelial defect indicates that it is due to the expression of a single recessive gene. This was borne out by experiment matings made for the special purpose of testing the matter. The conditions of the observations and experiments are such that good 3:1 ratios could not be obtained, but there is every indication that one is dealing with a simple monohybrid relationship. The allelomorphic normal gene is completely dominant, heterozygous dominants showing no visible evidence that they carry the defective heredity.

AUTHORS' SUMMARY.

RUPTURE OF HEART FROM ABSCESS. G. H. STEVENSON and A. J. MARSHALL, Glasgow M. J. **110**:337, 1928.

A boy, aged 9 years, died without warning from the rupture of the left ventricle through a myocardial abscess which had formed in the course of a pyemia of traumatic origin.

EXAMINATION OF BONE MARROW IN VIVO IN PERNICIOUS ANEMIA. L. FONTANA, Arch. per le sc. med. **52**:497, 1928.

The marrow from the body of the sternum was examined in twelve typical, mostly severe, cases of pernicious anemia. Megaloblasts were found in practically all cases; in seven from 2 to 6 per cent and in five from 10 to 21 per cent of the nucleated cells were megaloblasts. In view of the almost invariable presence of megaloblasts in the marrow in pernicious anemia and their absence in most other forms of anemia, examination of the marrow may have considerable diagnostic value. There seems to be no definite relation between the megaloblasts in the marrow and megaloblasts in the blood.

STUDIES OF GOITER IN MUNICH. HANS SPATZ, Deutsches Arch. f. klin. Med. **158**:257, 1928.

Spatz examined fifty-two goiters and thirteen normal thyroid glands and compared the microscopic, chemical and biologic data with the clinical symptoms. He found a direct relation between the clinical course and the efficiency of the goiter tissue in feeding tadpoles. Most of the goiters contain more iodine than normal thyroid glands. Iodine medication greatly increases the iodine content of the goiter. In adolescent and recurrent goiter, an extremely low relative and absolute iodine content is found.

Portions of the same goiter which show a greater biologic efficiency than the rest are usually richer in iodine. The microscopic picture does not run parallel with the clinical course, biologic efficiency and iodine content. Only the primary and secondary exophthalmic goiter, except the toxic adenoma, has a typical structure, strong biologic efficiency and low iodine content. The histologic picture of goiter with moderate hyperthyroidism often resembles that of exophthalmic goiter, but it cannot be distinguished sometimes from simple goiter.

The amount of colloid is always small in severe exophthalmic goiter, but it does not run parallel with the clinical, biologic and chemical data. Various portions of a gland can show so much difference in microscopic picture and biologic efficiency that only the examination of the whole gland is of value.

C. A. HELLWIG.

BONE MARROW CHANGES IN EXPERIMENTAL LEAD POISONING. J. SPERANSKY and R. SULIANSKAJA, Folia haemat. **36**:289, 1928.

In chronic lead poisoning the authors found that the bone marrow in the poisoned guinea-pig shows erythropoietic and leukopoietic changes. When the animals are treated with doses ranging from 38 to 70 mg. of lead per kilogram of weight, an increased number of erythroblasts and microblasts appear in the marrow. There also is a marked increase in the number of the young unripe cells, i. e., macroblasts and proerythroblasts. In instances in which there is a pronounced erythropoietic reaction in the marrow, this is followed by an extramedullary erythropoiesis (myeloid metaplasia of the spleen). There is a close relationship between the changes in the blood and those in the marrow. The authors were also able to notice that in normal guinea-pigs the morphology of the bone marrow is constant.

B. M. FRIED.

A CASE OF LYMPHOGRANULOMATOSIS MALIGNA COMBINED WITH TUBERCULOSIS. K. RUDSIT, Folia haemat. **36**:358, 1928.

Rudsit reports a case of a man, aged 50, who showed tuberculosis and Hodgkin's disease in the same glands. From a pathologic standpoint, there was a suggestion of an etiologic relationship between the two conditions. This case, therefore, would support the conception that malignant lymphoma is an "unusual" variety of tuberculosis. From a clinical point of view, the case is of interest in that it occurred in a person 50 years of age, which is uncommon; the Pirquet test was negative on repeated examinations; there were symptoms referred to the brain. The history of the thoroughly studied case is given in detail.

B. M. FRIED.

THE INTRAVASCULAR OCCURRENCE OF MEGACARYOCYTES IN MAN. SVEND PETRI, Folia haemat. **37**:129, 1928.

In three thoroughly studied cases of leukemic myelo-adenosis Petri found megacaryocytes in the blood smear, and also in the tissues removed post mortem. In one instance these cells were present in the renal vessels and in another they were conspicuous in the pulmonary capillaries. The so-called granular mega-

caryocytes contained red cells in their cytoplasm which he interprets as a phenomenon of phagocytosis. In no instance could he find what he calls "Wrightsche Figuren" whether in tissues or in dry smears, and he believes them to be artefacts. The article is from Fibiger's Institute and has a long list of references.

B. M. FRIED.

IODINE DEFICIENCY AND ENDEMIC GOITER. P. SCHMITZ-MOORMANN and F. MEIS, Mitt. a. d. Grenzgeb. u. Med. u. Chir. **41**:131, 1928.

In dogs, excision of one and one half of the thyroid is followed by compensatory hyperplasia of the remaining thyroid tissue. Goitrous glands show cellular proliferation only after resection of more than one and one half of the gland. By the administration of iodine, postoperative hyperplasia of the thyroid can be prevented. These experiments prove that endemic goiter is a compensatory process dependent on a relative or absolute deficiency of iodine.

C. A. HELLWIG.

THE HYPERFUNCTION OF THE ISLANDS OF LANGERHANS IN DOGS. D. E. ALPERN and W. P. BESUGLOW, Klin. Wchnschr. **7**:586, 1928.

The pancreatic duct was ligated, or a mass ligation was made through the tail of the pancreas. By these operations the external secretion of the gland was not completely interrupted and the digestion was not disturbed.

Changes resulted in the metabolism of carbohydrates, fats and mineral salts which were interpreted as the increased internal secretion of the pancreas and which resembled the changes seen after the injection of insulin. There was hypertrophy of the islands of Langerhans.

C. A. HELLWIG.

THE ORIGIN AND PRODUCTION OF RENAL CASTS. H. VON HOESSLIN, Klin. Wchnschr. **7**:1893, 1928.

A general review of the origin of renal casts is followed by a report of the structures formed in kidneys by maceration with hydrochloric acid and pepsin. Although epithelial and granular casts can be simulated by this method, the origin of hyaline casts is not so easily determined. Possibly they arise from slowly eliminated cell constituents. The origin of waxy casts is even more difficult to determine, but undoubtedly they also are derived from cell substance.

E. F. HIRSCH.

PATHOLOGY OF COLLAGENIC CONNECTIVE TISSUE FIBERS. S. WAIL, Virchows Arch. f. path. Anat. **267**:1, 1928.

Autopsies on two patients dying of sepsis revealed unusual changes in the collagenic fibers. These structures took the hematoxylin stain avidly, and presented a broken appearance, due to irregularity in staining. The pale central core of the fiber was surrounded at intervals by deeply stained rings. The same changes were produced in guinea-pigs by intraperitoneal injection of organisms from one of the human cases.

B. R. LOVETT.

AN ABNORMAL RIDGE IN THE LEFT VENTRICLE. L. BRINGS and A. SPITZER, Virchows Arch. f. path. Anat. **267**:9, 1928.

At autopsy on an infant with congenital heart disease, a connective tissue ridge was found in the left ventricle, on the boundary between the muscular and the membranous portions of the septum. It extended also on to the aortic leaflet of the mitral valve. An explanation for the development of this anomaly is given.

B. R. LOVETT.

PRIMARY BLOOD-FORMING HEMANGIO-ENDOTHELIOMA OF THE LIVER. G. ORZECHOWSKI, Virchows Arch. f. path. Anat. **267**:63, 1928.

Primary hemangio-endotheliomas of the liver are rare, malignant tumors, composed of endothelium, which attempts to perform its function of building blood vessels in the tumor mass. Two forms have been described, with and without formation of blood cells. In the author's case, tumor nodules were found throughout the liver, with newly formed blood vessels, and containing early forms of blood cells: myelocytes, metamyelocytes and nucleated red cells.

B. R. LOVETT.

"BLOCKADE" OF THE RETICULO-ENDOTHELIAL SYSTEM. G. L. DERMAN, Virchows Arch. f. path. Anat. **267**:73, 1928.

The author states that, following intravenous injections of ferri oxydum saccharatum into normal dogs and rabbits, the iron is found almost entirely in the reticulo-endothelial cells, in the liver, spleen and bone marrow. After a moderate degree of blockade of the reticulo-endothelial system, through several daily injections of collargol, the faculty of these organs to absorb iron is reduced, especially in the bone marrow. A higher degree of blockade calls forth proliferative activity, and the power of taking up iron is thereby increased. Splenectomy is followed by degenerative and proliferative changes in the reticulo-endothelial tissue, and in the bone marrow by increased activity in taking up iron, both with and without the blockade. The activity of the cells of the liver in this respect depends on the character of the degenerative and proliferative changes in that organ.

B. R. LOVETT.

RETICULOSIS AS A SYSTEMIC DISEASE OF THE BLOOD-FORMING ORGANS. T. TSCHISTOWITSCH and O. BYKOWA, Virchows Arch. f. path. Anat. **267**:91, 1928.

An unusual disease of the hemopoietic organs is described, presenting an isolated increase in the reticulo-endothelial elements of various lymph nodes, the spleen and in part of the bone marrow. Numerous "reticulomas" were found in the liver. The authors regard this as the first pure case of aleukemic reticulo-endotheliosis.

B. R. LOVETT.

SYSTEMIC DISEASE OF THE BONE MARROW. F. BATTAGLIA, Virchows Arch. f. path. Anat. **267**:106, 1928.

The process in myeloma is almost similar to that in the myeloses, but it presents certain differences, chiefly the destruction of bone, and the fact that it remains confined to the skeleton. The growth of nodules in the skeleton as a differential point appears to the author to be of little significance, since, as in his case, this process may be scarcely indicated. Cases of myeloma occur with leukemic blood changes, as well as with aleukemia. The significant factor seems to be the growth of the original hyaloid cells. The composition in the different cases depends on the period of development in which the cells are arrested and the cell type toward which they are developing.

B. R. LOVETT.

THE STRUCTURE OF ERYTHROCYTES. M. GUTSTEIN and G. WALLBACH, Virchows Arch. f. path. Anat. **267**:144, 1928.

Heinz, and later Ehrlich, described round bodies in the red cells, which appeared after treatment with a hemoglobin toxin, and which were presumably degeneration products containing methemoglobin. From a variety of staining reactions and the demonstration of the bodies without previous action of a toxin, the authors concluded that the bodies do not contain methemoglobin or any other hemoglobin degeneration product, and that their presence is not due to intoxication. They appear to be constituents of the normal red cell.

B. R. LOVETT.

LYMPH NODES IN ACUTE AND CHRONIC INFECTIONS. M. NORDMANN, Virchows Arch. f. path. Anat. **267**:158, 1928.

Systematic examination of lymph nodes in man enabled the recognition of two types according to histologic structure: (1) mesenteric and aortic nodes and (2) peripheral nodes (axillary and inguinal). The first type is characterized by prominence of the lymph sinus and marked development of the sinus endothelium. This depends on the large quantity of material for storage brought by the lymph stream, and is in relation to active metabolism in the adjacent organs. In the second type storage takes place in the reticulum of the lymphatic tissue without marked participation by the sinus. Storage of coal dust serves as an example. From these groups, one can deduce two general types of lymph node changes: (1) nodes with feeble absorbtive activity and storage in the reticulum of the lymphatic tissue and (2) nodes with strong absorbtive activity and storage in the increased endothelial and reticulum cells of the sinus.

Corresponding to the normal, under pathologic conditions of greatly increased material for storage, marked growth of the sinus endothelium is found, "sinus catarrh." This condition corresponds, therefore, to pathologically heightened metabolism in the regional organs, and not to local inflammation, or lymphadenitis. Storage in lymph nodes depends on the lymph flow, and occurs where the flow is slowest, or even stagnant, that is, in the lymphatic tissue of weakly absorbing glands and in the sinus of strongly absorbing glands. The different conditions of lymph flow explain the differences between the medulla and cortex, and also the histologic structure of passively congested glands. Lymphovascular induration is the late stage of sinus catarrh, while reticular induration of the lymphatic tissue represents the end-stage in the glands of peripheral type. Acute inflammatory processes, hyperplasia, necroses and consequent induration do not rest on functional alterations in the lymph stream, but are related to changes in the blood channels. In lymph nodes in acute and chronic general infections, sinus catarrh, in its various stages, plays the chief part, beside the inflammatory and hyperplastic changes.

B. R. LOVETT.

CELLULAR PROCESSES IN ATROPHY OF BONE. P. GUNKEL, Virchows Arch. f. path. Anat. **267**:204, 1928.

Atrophic changes in bone are called forth by altered cell activity, and destruction takes place only through the cells, and not through chemical activity of the tissue fluids. All destructive cells (osteoclasts, etc.), and perhaps also the building cells, originate from the blood vessels. The change of blood vessels into destructive cells begins in the periosteum, haversian canals and medulla with congestion and growth of the adventitia. The histologic picture is fundamentally the same in all atrophies, and shows differences only in that the process in disease of the circulatory system is slower, while in cachexia the early development of destructive elements is more prominent. Formation of osteoblasts takes place at the same time, but is soon overshadowed by the destroying activity. Fibrous atrophy occurs only in severe cases, especially in disease of the organs of circulation.

B. R. LOVETT.

MALFORMATIONS OF BOWMAN'S CAPSULE. E. RISAK, Virchows Arch. f. path. Anat. **267**:222, 1928.

Risak observed that in the kidneys of a woman dying with pyelonephritis, the parietal layer of Bowman's capsule in many glomeruli was composed of cuboidal epithelium similar to that of the proximal convoluted tubules. In some glomeruli the visceral layer was also of this type, and in one the cells were high and cylindric. In all, the capillaries were plump and the loops few. Study of fetal and infantile kidneys revealed that in embryologic development, the capsule epithelium is first of cylindric or cuboidal type, later becoming flattened. The author explains the abnormality in his case, not as a metamorphosis due to the chronic infection, but as a malformation due to arrested development.

B. R. LOVETT.

SYMPATHETICOTROPIC CELLS IN THE OVARY. L. BERGER, Virchows Arch. f. path. Anat. **267**:433, 1928.

Berger found the cells which he called "sympatheticotropic" 168 times in 218 pairs of ovaries. He denied the chromaffin nature of these cells (affirmed by Neumann), and found them similar in form and structure to the Leydig cells in the testis. Since they appeared to be of secretory nature and to bear some relation to the nervous mechanism of the organ, he referred to them as "neurocrine" structures.

B. R. LOVETT.

THE ETIOLOGIC CONDITIONS FOR THE DESTRUCTION OF HARD PARTS. B. ORBAN and J. WEINMANN, Virchows Arch. f. path. Anat. **267**:446, 1928.

From studies on the teeth of rats, it appeared improbable that narrowing of a cavity through building up on one side could result in absorption on the other side. The fact that absorption of uncalcified substance proceeds more slowly than that of calcified material probably depends on different characteristics of the tissue in question.

B. R. LOVETT.

LEUKEMIC RETICULO-ENDOTHELIOSIS. B. SWIRTSCHEWSKAJA, Virchows Arch. f. path Anat. **267**:456, 1928.

The author adds a case of monocytic leukemia to those already described in the literature. The clinical observations were those of a leukemia. The blood revealed from 50 to 96.2 per cent of cells of the monocytic type. At first they resembled adult monocytes, and later earlier forms of these cells, probably promonocytes. Histologically, there was found in the spleen atrophy of lymphoid tissue, areas of necrosis, increase in the connective tissue stroma, and changes in the sinus endothelium indicating a desquamation of endothelial cells, which subsequently multiplied in the blood and other organs. The case was, therefore, a monocytic leukemia, resting on hyperplasia of the reticulo-endothelial system, especially in the spleen, and to some extent in the liver and bone marrow. The author divides the cases in the literature into two groups: hyperplasia of the reticulo-endothelial system with relatively normal blood, and hyperplasia with monocytic blood, usually accompanied by myeloid hyperplasia in the bone marrow and elsewhere. His case suggests the possibility of the endothelial origin of the blood monocytes.

B. R. LOVETT.

EXPERIMENTAL STORAGE OF FAT. I. MARGAT, Virchows Arch. f. path. Anat. **267**:477, 1928.

Parenteral injection of lecithin in the form of an emulsion into guinea-pigs resulted in storage of the fat in the spleen, liver and lungs. This was more marked in tuberculous than in normal animals, not only in the region of tuberculous foci, but also in organs free from infection, such as the liver.

B. R. LOVETT.

CONTRIBUTION TO THE KNOWLEDGE OF INFLAMMATION AND OF MONOCYTES. M. SILBERBERG, Virchows Arch. f. path. Anat. **267**:483, 1928.

The first series of experiments on the effect of injections of benzene into rabbits showed that benzene acts as a selective toxin for the myeloid tissue, resulting in atrophy of the bone marrow and marked reduction of polymorphonuclear leukocytes in the blood, without much effect on the red cells or lymphocytes. In animals rendered nearly free from leukocytes in this way and exposed to septic infection, numerous necroses appeared in the organs, without inflammatory reaction or abscess formation. The macrophages were seen to be active. However, it was not observed that any other cells, particularly those of histiogenic origin, could take the place of the missing blood leukocytes, which are of the greatest importance in combating septic infection.

Experiments in vitro, with tissue from animals poisoned with benzene, revealed that the reticulo-endothelial system is entirely independent of the myeloid and lymphoid tissue, and is injured neither morphologically nor functionally by benzene. Derivation of monocytes from histiocytes could be shown in vitro, indicating the independence of these cells and their difference from the other blood cells. No transition between monocytes and lymphocytes or myeloid cells could be observed. In animals previously given injections with carmine particles so that the macrophages were "blocked," the course of infection was more severe than in control animals, and fewer organisms were taken up by the macrophages. The activity of the leukocytes was not impaired. The author believes in the independence and functional difference of the three types of white blood cells, leukocytes, lymphocytes and monocytes. Transition between the types, or derivation of one from another, is not possible in postembryonic life, although the three are embryologically derived from the same undifferentiated mesenchymal cell.

B. R. LOVETT.

CLASSIFICATION OF ENDOCARDITIS. H. KRISCHNER, Virchows Arch. f. path. Anat. **265**:545, 1927.

Krischner investigated seventy-eight cases of valvular disease at autopsy, and classified them according to the method of Beitzke. He distinguished simple or verrucous endocarditis and septic endocarditis, including polypous, ulcerative and mixed forms. In thirty-six cases of mixed endocarditis, thrombi were found only on the free borders of the leaflets, not exceeding a pinhead in size. They were pale yellow or reddish, and firmly adherent. Microscopically, the nodules consisted largely of blood platelets, without bacteria, fibrin or appreciable numbers of leukocytes. In rheumatic endocarditis, midway between the simple and septic types, the deposits were more extensive and larger, with cellular reaction extending into the substance of the leaflets, but with no serious anatomic defects resulting. Thirty-five cases of endocarditis polyposa showed depositions spreading beyond the border into the substance of the leaflets, and sometimes into the chordae tendinae. The nodules were larger than those observed in the simple form, reddish brown or reddish yellow, and either soft and friable or calcified. They consisted of a fibrinous network, containing leukocytes, red blood cells and bacteria, resembling a diphtheritic membrane. The leaflets were thickened, vascularized and sometimes revealed areas of necrosis surrounded by leukocytes. Only one instance of the ulcerative form was found. The cases of endocarditis simplex corresponded to a reparative inflammation, endocarditis rheumatica to a productive, and endocarditis polyposa or ulcerosa to a fibrinopurulent type.

B. R. LOVETT.

THE ORIGIN OF LEUKOCYTES IN ACUTE INFLAMMATORY EXUDATES. W. GERLACH and A. JORES, Virchows Arch. f. path. Anat. **267**:551, 1928.

Möllendorff's experiments on the excised jugular veins of guinea-pigs were repeated. Various irritants failed to produce any changes in the vessel walls or surrounding tissue suggestive of local formation of leukocytes from these tissues. Further experiments on animals rendered aleukocytic by means of injections of benzene failed to show evidence of local formation of leukocytes in response to irritants. All results indicated the blood stream, and originally the bone marrow, as the sole source of leukocytes in acute inflammatory reactions.

B. R. LOVETT.

CONGENITAL NONSPECIFIC PNEUMONIA AND PNEUMONIA FROM ASPIRATION IN THE BIRTH PASSAGES. H. HOOK and K. KATZ, Virchows Arch. f. path. Anat. **267**:571, 1928.

In an investigation of stillbirths and infants dying in the first few days of life, nonspecific aspiration pneumonia was found to be a frequent cause of death.

In seventy-three autopsies, congenital pneumonia was present twenty-two times and pneumonia in the first few days of life fourteen times. The presence of pneumonic foci could be determined in many cases only with the microscope. Amniotic fluid or other constituents of the birth canal were found regularly in the lungs. The pneumonias were of bronchogenic origin, even the congenital cases, due to aspiration of infectious material; there was no evidence of blood infection through the placenta. Histologic examination of the lungs should be made more frequently in these infants, especially in those dying with signs of asphyxia.

B. R. LOVETT.

ANEURYSM OF PLACENTAL ARTERY. O. HINTZE, Ztschr. f. Gynäk. **52**:2524, 1928.

Hintze reports two cases. Above the aneurysm, which was as large as a cherry, there was, in each case, a sharp kink in the artery and in one case the artery ran a serpentine course in its entire length. The artery affected was in each case that which supplied the larger part of the placenta. The aneurysm lay below the anastomosis, which was by a ramus intermedius. Birth was spontaneous; in one case there was premature detachment of the placenta and the child was born asphyxiated. The origin of the aneurysms could not be explained. Syphilis was not present. Hintze suggests that in unexplained intra-uterine death, the possibility of rupture of an aneurysm of a placental artery should be remembered.

SPASTIC PSEUDOSCLEROSIS. RICHARD ZIMMERMAN, Ztschr. f. d. ges. Neurol. u. Psychiat. **116**:1, 1928.

This seventh case of Jakob's disease revealed microscopically a diffuse and progressive process consisting of the complete disappearance of ganglion cells in small areas. The pyramidal cells either were sclerosed or revealed fatty and degenerative changes. The disease affected the entire cerebral cortex, especially the frontal and temporal lobes and motor cortex, although the basal ganglions were also much involved. It is interesting to note the author's attempt to correlate each clinical symptom with certain degenerated areas.

ROY GRINKER.

A CEREBELLAR CYST COMPLICATED BY CHRONIC ARACHNOIDITIS. L. M. GLAUBERMAN, Ztschr. f. d. ges. Neurol. u. Psychiat. **116**:15, 1928.

In a patient who had an apparently congenital cerebellar cyst without a tumor in its wall or any other possible explanation for its presence, a rather mild trauma caused the development of a serous meningitis. This resulted in the formation of a second encapsulated cyst in the posterior fossa cyst. Repeated lumbar punctures relieved symptoms of intracranial pressure caused by the serous meningitis but resulted in an aseptic purulent meningitis which caused death.

ROY GRINKER.

BRAIN SWELLING. SUZANNE ZINGG, Ztschr. f. d. ges. Neurol. u. Psychiat. **116**:71, 1928.

A condition diagnosed as cerebral tumor of undetermined localization revealed, on postmortem examination, only a swelling of the brain (Reichardt). The patient was a woman, aged 17, and the brain weighed 1,420 Gm., about 200 Gm. overweight. No gross or microscopic cause for the increased weight could be found. The author therefore believes that the volume of the brain was greater than the capacity of the skull which, unfortunately, he neglected to measure.

ROY GRINKER.

INFECTIOUS TOXIC MYELITIS WITH DESTRUCTION OF THE SPINAL CORD. J. SILBERMANN, Ztschr. f. d. ges. Neurol. u. Psychiat. **116**:140, 1928.

Many acute infectious diseases have caused severe damage to the spinal cord which has been attributed to the action of a toxin, to the actual invasion of the cord by bacteria or to an involvement of the vascular system, secondarily affecting the cord. Trauma and syphilis have also been ascribed rôles as predisposing factors. The case reported had three contributing factors in that the patient, of syphilitic parents, fell on his back and developed a severe angina and gingivitis at the same time. Severe symptoms developed in the spinal cord a week later. At postmortem examination, one and one-half years after the injury, the entire lumbosacral cord was found destroyed without a trace of the normal nerve fibers. In the dorsal and cervical regions, added to the ascending secondary degeneration there were many small areas of softening. No definite decision was made as to the primary cause.

ROY GRINKER.

THE PATHOLOGY OF BRONCHIAL ASTHMA. HILDING BERGSTRAND, Acta path. et microbiol. Scandinav. **5**:251, 1928.

The results of the postmortem examination in two cases of bronchial asthma are described. The changes were not such as usually are caused by bacteria but are regarded rather as expressions of a general allergy at the same time as the asthma which in both the cases probably resulted from respiratory infection. The bronchi did not contain any exudate but the products of secretion of a hyperplastic lining, the epithelium cells of which were changed into goblet cells. The inflammatory fossae in the lungs were not characteristic of bronchitis or pneumonia but resembled in part the changes that result in the skin on the reinjection of protein into sensitized animals.

SYMPATHETIC GANGLIONEUROBLASTOMA. E. BUSCH, Acta path. et microbiol. Scandinav. **5**:289, 1928.

A case with metastases is reported in a woman aged 30 years. This case is stated to be the sixth of its kind to be recorded. The five previously reported cases are reviewed briefly.

Pathologic Chemistry and Physics

BILIARY ACIDS IN JAUNDICE. I. KATAYAMA, Arch. Int. Med. **42**:916, 1928.

Quantitative studies were made of biliary acids in the blood and urine in various diseases of the biliary tract, and in other miscellaneous diseases. Biliary pigments in the blood were followed by the van den Bergh test, and the icterus index. The average biliary acids content of normal blood serum was 7 mg. per hundred cubic centimeters, with no biliary acids in normal urine. Biliary acids appeared in the urine when the blood serum value reached 20 mg. Patients with cholecystitis, disease of the liver, catarrhal jaundice, obstructive jaundice, cardiac decompensation and duodenitis showed a marked increase of biliary acids in the blood serum, accompanied by excreted biliary acids in the urine.

HAMILTON R. FISHBACK.

THE MANGANESE-COPPER-IRON COMPLEX AS A FACTOR IN HEMOGLOBIN BUILDING. R. W. TITUS, H. W. CAVE and J. S. HUGHES, J. Biol. Chem. **80**:565, 1928.

Manganese added to a milk-iron diet seems to give almost, if not quite, as good results in the building of hemoglobin as does copper added in the same way. Manganese and copper added to a milk-iron diet appear to produce a quicker response from the standpoint of building of hemoglobin than does either copper or manganese when fed alone as a supplement. Experimental data presented seem to indicate the existence of a group of substances, rather than a single substance, which is active in the building of hemoglobin.

AUTHORS' SUMMARY.

A Comparison of the Thresholds of Ketosis in Diabetes, Epilepsy and Obesity. W. S. McClellan, H. J. Spencer, E. A. Falk and E. F. Du Bois, J. Biol. Chem. **80**:639, 1928.

The threshold of ketosis is defined as the fatty acid dextrose ratio characterizing the food being metabolized at the moment when an abnormal concentration of acetone substances first becomes apparent in the urine. Studies of six men—three of them normal, one epileptic, one diabetic and one obese—indicate that while the threshold may be reached normally at ratios of 1:1.5, it may be somewhat higher in diabetes and epilepsy and considerably higher in obesity.

Arthur Locke.

Changes in the Rate of Excretion of Acetone Bodies During the Twenty-Four Hours. W. S. McClellan and V. Toscani, J. Biol. Chem. **80**:653, 1928.

In persons manifesting ketosis, acetone substances appear in the urine in greatest concentration during the later afternoon and night.

Arthur Locke.

Bile Salt Metabolism. G. H. Whipple and H. P. Smith, J. Biol. Chem. **80**: 659, 671, 685 and 697, 1928.

Neither cholesterol, yeast nucleic acid nor the alcoholic extractives of meat tissue appear to influence the rate of synthesis and secretion of the biliary acids. The contrasted, marked acceleration produced following the ingestion of whole meats and such meat derivatives as retain a moiety of protein suggests that the biliary acids are synthesized from products of protein disintegration rather than from lipid and cholesterol, as has been assumed.

Arthur Locke.

Biometry of Calcium, Inorganic Phosphorus, Cholesterol, and Lipoid Phosphorus in the Blood of Rabbits. Alvin R. Harnes, J. Exper. Med. **49**:287, 1929.

A series of determinations of inorganic phosphorus, calcium, cholesterol and lecithin were made on a group of ten animals living in the laboratory from Oct. 27, 1927, to May 17, 1928. A marked difference in both the trend and the absolute values was noted in animals living in the laboratory when compared with the values obtained for animals living out-of-doors. With animals living in the open, the trend of variation for calcium was found to be the same. However, animals living in the laboratory maintained a higher level over the same period of time. The inorganic phosphorus and lecithin both showed a marked decrease and both maintained a lower level than was found in animals just received from the dealer. The cholesterol content of whole blood exhibited a similar trend in both groups of animals. However, it was found that animals living in the laboratory maintained a lower level than animals living out-of-doors. The mean value for calcium was found to be 15.7 ± 0.05 and for inorganic phosphorus 4.65 ± 0.05 mg. per hundred cubic centimeters of blood serum, and for cholesterol 58.2 ± 0.39 and for lecithin 118.4 ± 1.13 mg. per hundred cubic centimeters of whole blood. The coefficients of correlation having the highest degree of mathematical significance were obtained from the mean values for individual animals.

Author's Summary.

Melanotic Pigments. R. L. Mayer, Klin. Wchnschr. **7**:2471, 1928.

The precursors of the pigment in melanin-producing cells, oxidized to quinones from substances such as tyrosin, epinephrine, etc., combine with cell protein to form the melanins. The melanin pigment therefore consists of a quinone and a combined protein group. Both may vary in composition. Lipoids or lipoid-like substances may participate in the second group.

Edwin F. Hirsch.

Microbiology and Parasitology

THE CLINICAL SIGNIFICANCE OF THE LIFE CYCLE OF THE PARASITE IN INDUCED MALARIAS. NICHOLAS KOPELOFF and CHARLES O. FIERTZ, Am. J. M. Sc. **176**:664, 1928.

A detailed microscopic study of the life cycle of a single strain of malaria inoculated intravenously into 300 patients with general paralysis reveals a total absence of gametocytes. The asexual cycle of this strain is identical with that of mosquito malaria. Such a strain is of practical value in the treatment for general paralysis in that: (*a*) it eliminates the possibility of the transmission of malaria to other members of the community, and (*b*) it precludes the occurrence of malarial relapse following adequate administration of quinine.

PEARL ZEEK.

EXPERIMENTAL SUBCUTANEOUS RHEUMATIC NODULES. B. J. CLAWSON, Am. J. Path. **4**:565, 1928.

By injecting streptococci into the subcutaneous tissues of rabbits, lesions can be produced which are morphologically similar to the nodules found in the subcutaneous tissue in cases of acute rheumatic fever. Since these experimental nodules obviously occur as a result of injecting streptococci, the probable conclusion is suggested that acute rheumatic fever and the type of inflammation associated with it are of streptococcic origin.

AUTHOR'S SUMMARY.

THE REGENERATION OF ACID-FASTNESS IN APPARENTLY DEGENERATED TUBERCLE BACILLI. HENRY C. SWEANY, Am. Rev. Tuberc. **18**:630, 1928.

Two unusual strains of tubercle bacilli were studied under unfavorable conditions. Degeneration forms were observed and described. In addition to the forms reverting directly to acid-fast bacilli, there are some that appear to have degenerated to a temporary nonacid-fast condition. These organisms are sometimes slowly growing coccoid or bacillary forms that are capable of reverting rather quickly to typical tubercle bacilli. Sometimes they may be rapid-growing, granular, bacillary or diphtheroid forms that revert to typical organisms after one or more animal passages. In the return to their characteristic form these organisms regenerate by a gradual transition that appears to be simultaneous with a corresponding change in the pathologic features. The organisms appear to become gradually acid-fast, beginning in the granule, which is also the last to lose it on regeneration. Cultures were grown from the end-products of these experiments which resembled the human tubercle bacillus. The pathologic change produced was at first exudative but gradually passed over into a more proliferative type with "epithelioid" and giant cells. It is believed that these facts will help to explain some obscure clinical and bacteriologic observations in tuberculosis.

H. J. CORPER.

THE CULTIVATION OF THE TUBERCLE BACILLUS. HENRY C. SWEANY and MAX EVANOFF, Am. Rev. Tuberc. **18**:661, 1928.

The authors use 3 per cent sodium hydroxide for twenty minutes to destroy contaminators and concentrate the material. Whenever possible they inoculate directly without treatment because a caustic agent may be detrimental to a certain number of tubercle bacilli. Inhibiting substances have been practically abandoned, reliance being placed on a careful preparation of the material and a selective environment. Several culture mediums have been described, and two of them are recommended for use; one consists of veal egg medium, in which the veal is leached with sterile milk instead of water as in the Petroff method, and the other is like the first except that 10 per cent sterile cream is substituted for glycerin. More than 90 per cent of positive results were obtained consistently by these methods with less than 15 per cent of contamination. The cream medium is especially good for growing the bovine tubercle bacillus. In comparing these mediums with animal

inoculation, in which a series of twenty-four specimens, negative by direct smear, was used, ten were negative by all methods. Fourteen others, 85.7 per cent, were positive by culture, while 72.8 per cent were positive by animal inoculation. Although one animal was positive with contaminated culture, three cultures grew from specimens that were negative on inoculation into guinea-pigs.

H. J. CORPER.

THE FATE OF TUBERCLE BACILLI IN VARIOUS ORGANS. H. J. CORPER and NAO UYEI, Am. Rev. Tuberc. **18**:672, 1928.

Guinea-pig inoculation is a far more delicate test for the presence of tubercle bacilli in tissues following intravenous injection of these bacilli into dogs and rabbits than are stained sections for bacilli or the development of tubercle or pathologic tissue changes in the organs of these animals. The morphologic changes in the bacilli occurring during their destruction in the organs of rabbits and dogs are difficult of determination when virulent bacilli are being used, because the number of bacilli are too few to be satisfactorily discernible by staining methods for this purpose. Comparatively large numbers of dead or avirulent bacilli must be given, so that they can be observed in stained smears with the microscope. Such observations as have been made make it seem likely that tubercle bacilli undergo the same changes in vivo as occur during cytomorphosis in vitro. As gaged from guinea-pig inoculation of the organs of rabbits and dogs at intervals after the intravenous injections of nonlethal amounts of virulent human tubercle bacilli, these bacilli are actively destroyed in the various organs of the rabbit and dog, although at a variable rate. In general, however, while viable tubercle bacilli were found early after injection into the circulation primarily only in the important organs of deposition, namely, the lung, liver, spleen, bone marrôw and kidney, as these animals either succumb to the infection or survive, the bacilli would be found in the majority of the organs tested or would disappear following recovery from infection, leaving in many cases no appreciable histologic evidence of the presence of these bacilli except such as might occur in growing young animals, with impairment of the permanent parts of the body, such as the teeth and the bone. The animals given large amounts of bacilli usually pass through a symptomatic period of acute illness, which terminates either in recovery, with subsequent absence of disease, or a fatal outcome with generalized disease. In some cases, in which disease persisted or developed after a lapse of time, the lesions and presence of bacilli (as determined by guinea-pig inoculation) usually predominated in the organs previously reported from studies on organic tuberculosis as most susceptible to the disease, such as the lung of the rabbit and the liver and lung of the dog. The usual incidence of fatal outcome following intravenous infection was variable in the resistant animal given relatively large infecting doses, but the period lay between the first and the sixth month after infection, with the earlier months, following the first, being most consequential. After from six to nine months recovery was the rule.

H. J. CORPER.

TUBERCULOSIS MORTALITY IN THE ORIGINAL DEATH-REGISTRATION STATES. CORA E. GRAY, Am. Rev. Tuberc. **18**:687, 1928.

From this general study certain facts are noted: 1. The peak of the death rate in adult life is not, for the area as a whole nor for most of the states, moving toward the older ages. During the twenty-five years under study it remained in the twentieth to the twenty-ninth year group. 2. Although there is a relation in the years from 1900 to 1924 between the death rate from tuberculosis and that from all other causes, there is none in the years from 1900 to 1904, and there is no relation between the decreases in the two death rates in the years under study. Presumably, the factors which now influence the death rate for tuberculosis are not those which affect the death rate from other causes. 3. The states with the highest death rates at the beginning of the period have made the greatest decreases, so that the range in the death rate for tuberculosis at the end of the period is much less than at the beginning.

H. J. CORPER.

THE DEVELOPMENTAL CYCLE OF THE TUBERCLE BACILLUS AS REVEALED BY SINGLE CELL STUDIES. MORTON C. KAHN and JOHN C. TORREY, Am. Rev. Tuberc. **18**:815, 1928.

The results of studies of more than 200 preparations of single tubercle bacilli each in a separate microdroplet, under conditions which often ensured active growth and in such a manner as to maintain them in actively viable form for several days and often weeks, are recorded. Transplants were maintained on Long's synthetic medium with agar or gelatin. Branching forms, segmentation, globoid and coccoid forms are described. Nonacid-fast types were numerous.

H. J. CORPER.

THE FILTERABILITY OF THE TUBERCLE BACILLUS. WILLIAM P. THOMPSON and MARTIN FROBISCHER, JR., Am. Rev. Tuberc. **18**:823, 1928.

Acid-fast bacilli may be found in the lymph nodes of 35 per cent of normal guinea-pigs. These results do not warrant the assumption of an invisible form of tubercle bacillus capable of passing through a filter candle, and capable of infecting and killing guinea-pigs.

H. J. CORPER.

A POSSIBLE SOURCE OF SO-CALLED SPONTANEOUS TUBERCULOSIS IN GUINEA-PIGS. HENRY SEWALL, Am. Rev. Tuberc. **18**:829, 1928.

In establishments which harbor tuberculous patients and in which the animal caretakers may be tuberculous, meticulous precautions should be exercised to be sure that guinea-pigs kept for experimental purposes are maintained in an uncontaminated environment; the food supply should have had no contact with the subjects of tuberculosis, nor, it may be assumed, should persons with tuberculosis be allowed to care for them. If children were studied instead of guinea-pigs it will be admitted that the conditions under suspicion would sufficiently explain the advent of alimentary and contact tuberculosis.

H. J. CORPER.

THE RELATION OF PLASMODIUM FALCIPARUM TO THE HUMAN RED BLOOD CELL AS DETERMINED BY SECTIONS. HERBERT L. RATLIFF, Am. J. Trop. Med. **8**:559, 1928.

Placental tissues from a person infected with *Plasmodium falciparum* were fixed in Bouin's fluid, dehydrated and embedded in paraffin. Sections 2 microns thick were cut and stained in Harris' hematoxylin. The parasites have been found invariably to be intracellular.

AUTHOR'S SUMMARY.

UNDULANT FEVER IN CONNECTICUT. T. P. MURDOCK and W. E. HALL, Ann. Int. Med. **2**:545, 1928.

Three cases of undulant fever were discovered by the writers in Meridan, Connecticut. The serum agglutinated *B. abortus* to 1:300.

WALTER M. SIMPSON

THE DOG A RESERVOIR OF THE BROAD TAPEWORM. TEUNIS VERGEER, J. A. M. A. **92**:607, 1929.

Broad tapeworm is common in the wall-eyes and great northern pikes of the large Canadian lakes. The fish in Lake Nipigon apparently are the most heavily infested. The adult worms have been brought over from Europe by infested Scandinavian, Finnish and Russian immigrants.

In some small lakes in lumbered forest regions, from 50 to 75 per cent of the wall-eyes and great northern pikes are infested.

The lumbering concerns employ Scandinavians and Finns almost exclusively; they cause the original infestation of fish in the small lakes, and as lumbering operations advance they move on.

Indians and others feed their dogs on raw wall-eyes and great northern pike; the dogs become an important reservoir of broad tapeworm and reinfest the fish in the lakes. Dogs are capable of harboring from five to nineteen broad tapeworms.

Fish in lakes near Indian reservations are most heavily infested. Three large Indian reservations are located on the shores and islands of Lake Nipigon with another reservation on the Nipigon river, which probably accounts for the heavy infestation in the Nipigon fish.

Indian agents and teachers should be instructed to teach the Indians the necessity of cooking fish even for dogs for the sake of dog, fish and man.

Lumbermen and their families in lumber camps should be inspected for tapeworm and treated if infested.

The importation of adult tapeworms by immigration should be prevented.

AUTHOR'S SUMMARY.

MATT AND GLOSSY FORMS OF HEMOLYTIC STREPTOCOCCI. E. W. TODD and R. C. LANCEFIELD, J. Exper. Med. **48**:751 and 769, 1928.

Hemolytic streptococci, when freshly isolated from pathogenic lesions, form characteristic matt colonies and contain the type-specific substance M. Two varieties of matt cultures, equally rich in type-specific substance, can be distinguished by the virulence of the organisms for mice: the matt virulent variety, the matt attenuated variety. The matt forms of hemolytic streptococci can be degraded to a third variety which forms glossy colonies and is always relatively avirulent. This is accomplished by prolonged cultivation on artificial mediums, by selection of colonies or by cultivation in homologous anti-M serum. In the process of degradation, the cocci lose the major part of their type-specific substance, but complete disappearance of type-specific substance rarely occurs. The glossy variant form, when fully degraded, is highly stable; but glossy cultures which have retained some type-specific substance can occasionally be reverted to the original matt form. Toxic filtrates from matt and glossy cultures are approximately equal in skin reactivity. No relationship appears to exist between virulence and toxigenicity.

The matt and the glossy forms of four strains of hemolytic streptococci were used to immunize rabbits. Precipitin tests showed that rabbit serums prepared against matt organisms, whether virulent or avirulent for mice, contained type-specific antibody, while serums prepared against completely degraded glossy organisms contained no type-specific antibody. Type-specific antibody was removed from the serums by absorption with homologous matt organisms but was unaffected by absorption with homologous glossy organisms. Passive protection experiments on mice showed that antimatt serums were protective and antiglossy serums non-protective against infection with homologous virulent organisms. Vaccination of mice with matt organisms rendered them immune to subsequent infection with homologous virulent cultures; but vaccination with glossy organisms established no active immunity.

AUTHORS' SUMMARY.

EXPERIMENTAL TYPHOID FEVER IN THE GUINEA-PIG. WILLIAM H. HARRIS and OGILVIE M. LARIMORE, J. Exper. Med. **48**:885, 1928.

During the activity of peritonitis produced in the guinea-pig by means of *Bacillus typhosus*, there is formed in the exudative material a filtrable toxic moiety which when inoculated into normal animals of this species produces certain of the clinical phenomena and a pathologic picture simulating that of human typhoid fever.

AUTHORS' SUMMARY.

A STATISTICAL STUDY OF SCARLET FEVER AND DIPHTHERIA. HILDA M. WOODS, J. Hyg. **28**:147, 1928.

The mortality from scarlet fever has declined relatively the most at the ages between 0 and 5 years, and there is a tendency for a greater proportion of the mortality to occur among older children and young adults.

The mortality from diphtheria appears to be concentrated on children of the early school age and decreased at older ages.

The decline in incidence and mortality from scarlet fever has been as great in towns with little isolation as in those in which the majority of patients are hospitalized.

It cannot be shown that during the period studied isolation has had either a good or bad effect on the prevalence or mortality of scarlet fever.

As far as the analysis goes and the method of correlation can show, there is no evidence pointing to the advantageous results of the isolation of patients with diphtheria in London.

AUTHOR'S SUMMARY.

A HEMOLYTIC SUBSTANCE IN PNEUMOCOCCUS CULTURE BROTH. G. M. SICKLES and J. M. COFFEY, J. Infect. Dis. **43**:490, 1928.

There is present in the culture broth of pneumococcus strains of different types and degrees of virulence a substance which has the property of lysing red blood cells. This substance was produced when pneumococci were grown in several different mediums and appeared after from four to twelve hours' incubation depending on the medium and the strain. The most potent substance was obtained when the pneumococcus strains were grown in beef-infusion broth plus 1 per cent dextrose, plus calcium carbonate with a small surface exposed to the air.

The hemolytic substance is thermolabile and is destroyed by heating for thirty minutes at 55 C. Its activity is diminished by standing at cold-room temperature and, in a shorter time, at 37 C. The hemolytic substance may be absorbed from the culture broth by sheep red cells, horse stroma, guinea-pig leukocytes, animal charcoal and alumina. Normal horse serum has an inhibitory effect on the hemolytic power of the substance while pneumococcus immune serum has a much more marked effect.

AUTHORS' SUMMARY.

STUDIES ON BOTULINUS TOXIN. E. WAGNER SOMMER and H. SOMMER, J. Infect. Dis. **43**:496, 1928.

On incubation of neutral botulinum toxin in salt solution at 37 C. a decrease in potency of from 90 to 99 per cent occurred in twenty-four hours. The deterioration was accelerated in alkaline mediums, but was greatly retarded at p_H 4.3. The addition of serum caused an immediate increase in toxicity; in several samples a further rise in the titer of the toxin was observed on incubation. Witte's peptone showed the same influence as serum but in a less marked degree. Dilution in a 20 per cent aminoid solution greatly stabilized the toxin. The addition of lecithin slightly increased the initial titer of the toxin. No effect was observed with sodium stearate and oleate. Sodium ricinoleate caused rapid though not immediate inactivation of the poison.

The results are discussed in the light of the protoxin and toxinase theories.

AUTHORS' SUMMARY.

A POISON PRODUCED BY BACTERIUM ENTERITIDIS AND BACTERIUM AERTRYCKE WHICH IS ACTIVE IN MICE WHEN GIVEN BY MOUTH. SARA E. BRANHAM, LUCILE ROBEY and LOIS A. DAY, J. Infect. Dis. **43**:507, 1928.

Seventeen strains of paratyphoid bacteria, isolated from foods or rodents, or from persons infected during food poisoning outbreaks, produced fatal infection in 100 per cent of mice to which they were fed. These strains included seven of *Bacterium enteritidis*, nine of *B. aertrycke* and one of *B. schottmülleri*.

When boiled broth cultures and Berkefeld N and W filtrates of broth cultures of these strains were fed similarly to mice, a mortality rate of approximately 40 per cent occurred.

When whole unfiltered cultures in beef heart medium are boiled or autoclaved, and then fed to mice, the mortality rate is often from 40 to 100 per cent.

Feeding autolyzed, boiled or autoclaved suspensions of the washed bacteria had little, if any, effect. Filtrates of twenty-four hour cultures produced a higher mortality rate than those from cultures which were incubated for longer periods of time.

In its remarkable heat stability this poison resembles the other toxic materials which have been described in the paratyphoid group, but its surprisingly long incubation period seems to separate this toxic substance definitely from that responsible for the violent gastro-intestinal symptoms occurring in man after eating foods containing these bacteria, and from any other toxic product of these bacteria that has yet been described.

Further investigation will be necessary before it can be determined whether this toxic principle is a product of the bacterial cells or a chemical poison formed by the action of the micro-organisms on the constituents of the medium.

AUTHORS' SUMMARY.

EXPERIMENTS WITH GUINEA-PIG VACCINIA VIRUS. J. O. W. BLAND, Brit. J. Exper. Path. **9**:283, 1928.

Vaccinia virus from lesions of the skin of guinea-pigs fails to pass collodion membranes which allow the passage of serum globulins. Treatment of such membranes with serum increases their permeability to protein. The virus can be thrown down by high speed centrifugation for from two to two and one-half hours. The particles with which this virus is associated are possibly within the limits of microscopic visibility.

PEARL ZEEK.

BACTERICIDAL POWER OF "WHOLE" BLOOD STUDIED BY CULTURE IN SLIDE-CELLS. J. M. ALSTON, Brit. J. Exper. Path. **9**:300, 1928.

The slide-cell method of testing the bactericidal power of whole blood is described, and is found to be reliable. Diurnal variations are found in the bactericidal power of the blood of healthy persons and animals. Other transient changes are produced by a wide variety of substances.

PEARL ZEEK.

THE EFFECT OF ULTRAVIOLET LIGHT ON THE VIABILITY OF THE VIRUS OF FOOT AND MOUTH DISEASE. I. A. GALLOWAY and A. EIDINOW, Brit. J. Exper. Path. **9**:326, 1928.

The virus of foot and mouth disease in filtrates is destroyed after five minutes' exposure to the radiations of the mercury vapour lamp in quartz flasks, which transmit radiations of wave lengths of from 5,720 to 2,300 Angstrom units; it is inactivated after thirty minutes' exposure to the rays of the mercury vapour lamp filtered through a sheet of vita-glass which allows the passage of rays of wave lengths of from 5,720 to 2,800 Angstrom units. The rays of wave lengths of from 5,720 to 2,300 Angstrom units have no lethal action on the virus of foot and mouth disease. When the virus is suspended in unfiltered lymph or serum, a protective action due to selective absorption by the suspending fluid is observed.

AUTHORS' SUMMARY.

BEHAVIOR OF THE BACTERIOPHAGE IN THE PRESENCE OF NONSOLUBLE MICRO-ORGANISMS. PAUL FABRY, Arch. internat. de méd. expér. **4**:413, 1928.

The bacteriophage does not multiply in the presence of nonsoluble micro-organisms. In certain cases, however, if a suspectible species of organism is added to the combination of bacteriophage and apparently nonsoluble micro-organism, the bacteriophage multiplies in the same manner as when added to a culture of readily soluble organisms, thus indicating that there may be a beginning of lytic action, though it is not apparent.

PEARL ZEEK.

UNITY OF THE SPIROCHAETES OF THE DUTTON GROUP. CHARLES NICOLLE and CHARLES ANDERSON, Arch. de l'Inst. Pasteur de Tunis **17**:321, 1928.

Three strains of spirochetes of the so-called Dutton group, from various sources, are shown to be identical. In view of the fact that the spirochetes are so widely distributed geographically and that they may reside in various hosts, the results are of importance in infection and in vaccination.

M. S. MARSHALL.

NOTE ON MOROCCAN RECURRENT FEVER. PIERRE HORNUS, Arch. de l'Inst. Pasteur de Tunis **17**:327, 1928.

Recurrent fever, observed in certain parts in Morocco, has a predilection for Europeans during the fall. The disease develops around piggeries. The parasite which transmits it seems to be found in the soil around habitations and retains its virulence for a considerable time. The spirochete, the etiologic agent, may be inoculated into guinea-pigs, in which a series of attacks are produced analogous to those in man.

AUTHOR'S SUMMARY.

FIXED VIRUS DOES NOT PASS INTO THE CENTRAL NERVOUS SYSTEM IN THE COURSE OF ANTIRABIC TREATMENT. P. REMLINGER and J. BAILLY, Ann. de l'Inst. Pasteur **42**:729, 1928.

The authors present experimental evidence in support of their title.

M. S. MARSHALL.

STUDIES OF FOOT-AND-MOUTH DISEASE. H. VALLÉE and H. CARRÉ, Ann. de l'Inst. Pasteur **42**:841, 1928.

There seem to be two races of the virus of foot-and-mouth disease, called O and A. Waldmann and Trautwein have added a third type (C). The only differentiation between the O and A types is based on immunity reactions, but these appear constant, the immunity from each being valueless against the other type. The O type is most common in France, England and Germany, but both types are found in various countries. Immunity following a first infection appears complete in both cases. Reinoculation under unknown conditions may bring about initial susceptibility instead of hyperimmunity.

AUTHORS' SUMMARY.

TRANSPLACENTAL INFECTION WITH TUBERCULOUS ULTRAVIRUS AND TUBERCULOUS HEREDITY. A. CALMETTE, J. VALTIS and M. LACOMME, Ann. de l'Inst. Pasteur **42**:1149, 1928.

The authors consider the transplacental transmission of the normal form of the tubercle bacillus to be infrequent. It was demonstrated in only three of twenty-six infants of tuberculous mothers. Transplacental transmission of the filtrable ultravirus is much more common. It appears particularly intense after from three to six months' gestation in women with developing tuberculosis, especially the pulmonary or meningitic type. Usually these are fatal to the child in the first weeks after birth. Then, since the death rate in the first two months of life of infants born of tuberculous mothers does not appear to exceed 20 per cent, and the number of children of tuberculous mothers born with the ultravirus is greater (perhaps 80 per cent), it may be concluded that perhaps nearly 60 per cent of the new-born carriers of the ultravirus support this infection without immediate harm. There seems to be some immunity. The matter is being studied. Thus far, the distinction between transplacental transmission of the tubercle bacillus and of the ultravirus does not in any way indicate a change from the procedure of separation from the mother or the use of B. C. G.

M. S. MARSHALL.

BACTERIOLOGY AND SEROTHERAPY IN ACUTE APPENDICITIS. M. WEINBERG, A.-R. PRÉVOT, J. DAVESNE and CLAUDIE RENARD, Ann. de l'Inst. Pasteur **42**:1167, 1928.

The authors believe that appendicitis is always associated with bacterial invasion. Of the aerobes, *B. coli* was found in 87 per cent of the cases and the enterococcus in 30 per cent. The most common anaerobe was *B. perfringens*, which was found in 30 per cent of the cases. In 41 of 160 cases, *B. coli* and the enterococcus were found associated with or without other organisms. In 51 of 160 cases, *B. coli* and *B. perfringens* existed together in the same specimens. Experiments with rabbits indicated that the latter combination might be most important in appendicitis. An antigangrene serum in which the anti-*oedematiens* fraction was replaced with anticolon serum was used with good results.

M. S. MARSHALL.

EXPERIMENTAL RESEARCH ON THE PATHOGENICITY OF CHOLERA. P. ZDRODOWSKI, Ann. de l'Inst. Pasteur **42**:1242, 1928.

The author confirms and extends Sanarelli's work. An anaphylactoid reaction, called by the author The Phenomenon of Sanarelli, follows a state of hypersensitivity produced by a homologous or heterologous protein. Lesions in various organs and tissues, primarily in the epithelial and endothelial system, resulting from this reaction pave the way for the invasion by otherwise often harmless organisms. This opens the way for a new conception of intestinal diseases, explaining perhaps the pathogenicity in Asiatic cholera and cholera nostras, and some forms of appendicitis and ulcerous processes in the intestines.

M. S. MARSHALL.

THE PATHOGENESIS OF RELAPSE IN EXPERIMENTAL RELAPSING FEVER. A. M. BRUSSIN and G. J. ROGOWA, Centralbl. f. Bakteriol. **105**:39, 1928.

After briefly reviewing the literature concerning the mechanism of relapse in recurrent fever, the authors describe experiments utilizing the "beladungs" phenomenon in the mouse. This consists in mixing the spirochetes with citrated blood from an immunized mouse; if the antibodies in the latter, called thrombocytobarines, are specific, the spirochetes will become covered with blood platelets. The technical features and advantages of the procedure are described. By means of this method, the authors confirmed the earlier observations of Levaditi and Roché and of Manteufel that the antigenic properties of spirochetes of the first and second relapses of African relapsing fever are changed; in addition they confirmed the observation of Rosenthal with regard to the same effect with spirochetes from European relapsing fever. After the second relapse, the ability of the spirochetes to acquire new antigenic properties lessened in most instances, and usually the latter were identical with those of the second relapse. Clinical cure depends on the accumulation of antibodies against both the infecting strain and the relapse variants.

PAUL R. CANNON.

THE DEMONSTRATION OF VACCINE VIRUS IN THE BLOOD AFTER CUTANEOUS INOCULATION. E. GILDERMEISTER and GEORG HEUER, Centralbl. f. Bakteriol. **105**:86, 1928.

The cutaneous injection of variola vaccine into rabbits was followed by its appearance in the blood within as short a time as two hours; it remained there for several days, as demonstrated by the method of Calmette and Guerin. The injection of a suspension of Pelikan ink (25 cc.) had no influence, the authors thereby concluding that blockading the reticulo-endothelial system has no influence on the demonstration of the vaccine virus in the blood stream.

PAUL R. CANNON.

A New Cause of Mouse Septicemia (Corynebacterium Murisepticum n. sp.). Gertrud Freiin von Holzhausen, Centralbl. f. Bakteriol. **105**:94, 1928.

The author describes an organism isolated from the brain of a rabid dog which regularly causes a fatal septicemia in mice. The organism is a gram-positive rod to which the name *Corynebacterium murisepticum* n. sp. is given. The bacterium is peculiar in that only the endothelial cells of the blood vessels appear to phagocytose it, the usual reticulo-endothelial cells remaining free.

Paul R. Cannon.

Rare Gram-Negative Anaerobic Bacilli as Unusual Causes of Acute Infections of the Middle Ear. Erich Wirth, Centralbl. f. Bakteriol. **105**:201, 1928.

Wirth describes anaerobic gram-negative bacilli isolated from two unusually severe infections of the middle ear. The organisms are pleomorphic, gas-forming and pathogenic for mice, guinea-pigs and rabbits. He thinks that they may be identical with *B. fundibuliformis*.

Paul R. Cannon.

The Changes in the Migration of Ascaris Larvae in the Body of the Host. G. G. Smirnow, Centralbl. f. Bakteriol. **105**:426, 1928.

The migration of *Ascaris* larvae in mice and guinea-pigs experimentally infected with embryonated eggs causes definite changes particularly in the wall of the cecum, in the liver and the lungs. The intensity and character of these pathologic changes depend on the degree of the invasion. The reaction in the compact tissues of the liver is less marked than in the porous tissues of the lungs. In response to the presence of the larvae there is an infiltration of eosinophils, polymorphonuclear leukocytes and a proliferation of macrophages, tending to encapsulate the larvae, with later fibrous changes and even calcification. The larvae in the liver enter through the portal vein and may enter the bile passages secondarily, but not primarily. The reactions in the lungs are predominantly mesenchymal. There is practically no invasion of other organs. Previous stimulation of the macrophages by vital staining appears to render the protection against the larvae more marked.

Paul R. Cannon.

The Transmission of Infectious Rat Anemia. Martin Mayer, Klin. Wchnschr. **7**:2390, 1928.

The rat louse, *Haematopinus spinulosus*, has been found to be a vector for infectious rat anemia.

E. F. Hirsch.

Experimental Syphilis of the Central Nervous System. I. L. Kritschewski and E. S. Heronimus, Klin. Wchnschr. **7**:2472, 1928.

The tropism of *Spirochaeta pallida* for the central nervous system is almost absolute, and various strains may not be classified into neurotropic and dermotropic.

E. F. Hirsch.

The Effect of Light Rays on Experimental Tuberculosis in Guinea-Pigs. H. Löwenstädt, Virchows Arch. f. path. Anat. **266**:99, 1927.

Guinea-pigs were inoculated subcutaneously with tubercle bacilli, and the effect of light rays on their general condition and on the histologic picture was observed. The controls presented a variable picture with regard to necrosis and encapsulation. In the spleen, demarcation of the tubercles took place, and in the lymph nodes

increase in the tubercles. None of the types of light rays used (carbon arc, mercury vapor, and alpine sun lamps) called forth a material difference in the process. One animal, which received repeated injections of eosin and was kept in weak diffuse light, showed a general reaction against tuberculosis, consisting of fibrous encapsulation of most necrotic foci. The author concluded that neither long nor short wave light rays influence the course of tuberculosis in guinea-pigs.

B. R. LOVETT.

PRIMARY TUBERCULOSIS OF THE MIDDLE EAR IN INFANTS. M. ZARFL, Virchows Arch. f. path. Anat. **266**:274, 1927.

The author investigated thoroughly four instances of primary tuberculous infection of the middle ear, in which the regional lymph glands were involved, and which presented the picture of a primary complex. Clinically, swelling of the auricular glands appeared first, followed by discharge from the ear, facial paralysis and swelling of the mastoid, with a positive tuberculin reaction in three cases. Pathologic investigation revealed indubitable tuberculous changes with the presence of tubercle bacilli in the structures of the middle ear, and failed to reveal a primary complex in any other part of the body. The infection apparently began in the mucous membrane, and spread to all nearby structures: the labyrinth, middle ear, facial canal, bony walls, etc. Infection probably entered through the eustachian tube. This could take place either during birth, through aspiration of infected amniotic fluid, or afterward from a person with open tuberculosis. In two of these cases the mother, and in one the father, suffered from tuberculosis; in the other, no source of infection could be found.

B. R. LOVETT.

VARIOLA-VACCINIA. HANS DEMME, Ztschr. f. Immunitätsforsch. u. exper. Therap. **55**:191, 1928.

The problem of neurotropism of the variola-vaccinia virus was investigated. Inoculations were made into the cornea, skin, testicle, sciatic nerve and subdural space of rabbits. Encephalitis was absent in all rabbits inoculated in peripheral organs. Pleocytosis of the spinal fluid following inoculation was interpreted as evidence of the participation of the central nervous system in the defensive reaction of the organism. There was no indication that the virus wanders by way of the nerves; on the contrary, the fact that the clinical course was the same after inoculation into the sciatic nerve, with and without previous surgical interruption, and the occurrence of cases of general vaccinia after inoculation of this nerve and the cornea point to the carriage of the virus by the blood. The encephalitis occurring after intracerebral inoculation was a nonspecific inflammatory reaction restricted to the meninges and vascular sheaths (mesenchymatous reaction) without participation of the ectodermal nervous substance. As these results differ from those after inoculation of herpes virus, variola-vaccinia does not belong to the neurotropic ectodermatoses. Its close relationship to herpes is denied. The vaccinia infection is interpreted as a general infection. It may activate a virus already present in the body or prepare the field for its activity. This interpretation applies also to human postvaccinial encephalitis.

WILLIAM C. HUEPER.

THE DESTINY OF TYPHOID BACILLUS INTRODUCED PER OS INTO DOGS. T. OUCHI, Scient. Rep. Gov. Inst. Inf. Dis. **6**:1, 1927.

The coprophagic habits of dogs when hungry may be an important factor in the extrahuman persistence of the typhoid bacillus. Human excrement when ingested by dogs passes directly into the bowel without stopping long in the stomach and hence does not receive the bactericidal action of the gastric juice.

E. P. JORDAN.

Immunology

THE TESTICULAR ALLERGIC RESPONSE IN GUINEA PIGS FOR THE DIAGNOSIS OF TUBERCULOUS FLUIDS. J. J. WIENER, JOHN E. BLAIR and HENRY L. JAFFE, Am. Rev. Tuberc. **19**:55, 1929.

In making a diagnosis, an attempt was made to apply Long's testicular tuberculin reaction to the body fluids suspected of coming from tuberculous foci. The test consists of the injection of from 0.1 to 0.3 cc. of the suspected fluid into the testis of a tuberculous guinea-pig, autopsy on the animal after from five to seven days, and the histologic examination of the testis for a characteristic degeneration. The diagnostic value of the test is impaired by the fact that about 35 per cent of nontuberculous fluids (particularly fluids from cases of malignant tumor) gave a reaction which was histologically indistinguishable from that given by tuberculous fluid. Data obtained show that this is a toxic effect, which apparently cannot be eliminated by reduction of the amount of fluid injected. However, if from 0.1 to 0.2 cc. of the fluid from a suspected tuberculous focus, with the exception of spinal fluids, does not produce the aforementioned reaction, on inoculation into the testis of a tuberculous guinea-pig, the possibility of tuberculosis of that focus may be definitely ruled out.

H. J. CORPER.

THE MODE OF ACTION OF FORMALDEHYDE ON COMPLEMENT FIXATION SYSTEMS. C. E. REYNER, J. Immunol. **16**:1, 1929.

The action of formaldehyde in intensifying the fixation of complement is due to its action on the antigen.

SURFACE TENSION OF BLOOD SERUM IN SYPHILIS. ELIZABETH M. YAGLE, J. Immunol. **16**:17, 1929.

Determinations of surface tension are of no value as a means of differentiating between syphilitic and normal serum. The so-called "syphilitic antibody," or reagin, is evidently not highly surface active or at least not more highly active than other substances found in normal and in syphilitic serum.

AUTHOR'S SUMMARY.

SERUM DILUTION AND ANTIGEN DENSITY AND THE NON-SPECIFIC PRECIPITATIONS OF FOWL SERUM. S. J. SCHILLING and G. S. SCHILLING, J. Immunol. **16**:61, 1929.

Increasing antigen density markedly decreases the nonspecific precipitation reaction in fowl serums. The most abundant precipitates are frequently found in dilutions of phenolized saline solution. This menstruum also regularly caused the precipitations in the highest dilutions of fowl serum. Grades *Salmonella pullorum* antigens of increasing densities showed a gradual fall in the highest dilution of fowl serum at which such respective antigens yielded precipitate. Evidence is adduced that a lipoidal element is responsible for the nonspecific precipitation reaction.

AUTHORS' SUMMARY.

VENOMS OF NORTH AMERICAN SNAKES AND THEIR RELATIONSHIP. THOMAS S. GITHENS and LEWIS W. BUTZ, J. Immunol. **16**:71, 1929.

The venoms of six species of North American rattlesnakes, which were studied, contain identical or almost identical toxic principles. The toxic principles of the copperhead and moccasin are identical or almost so, but differ to some extent from those of the rattlesnakes. The venom of *Crotalus terrificus* the dog-faced rattlesnake of Central and South America, and the venom of *Bothrops atrox*, the fer de lance, contain principles differing entirely from those of the Nearctic rattlesnakes as regards their antigenic properties. Rattlesnake venoms contain, in addition to their acutely toxic principles, other components which have the power to bind antibodies but which are not acutely toxic to pigeons.

AUTHORS' SUMMARY.

THE ABSENCE OF HETEROPHILIC ANTIGEN IN CERTAIN FOODS. I. DAVIDSOHN, J. Infect. Dis. **44**:44, 1929.

Twenty-six rabbits were given injections of watery suspensions of cabbage, lettuce, carrots, potatoes, wheat, corn and oats, with their alcoholic extracts and of a mixture of these alcoholic extracts and pig serum to determine whether the foregoing substances contain heterophilic antigen. The immunization was not followed by an increase of the antisheep hemolysin. Cabbage, lettuce, carrots, potatoes, wheat, corn and oats do not contain heterophilic antigen of the Forssman type.

AUTHOR'S SUMMARY.

ACID AGGLUTINATION OPTIMUM IN THE BRUCELLA GROUP. E. E. ECKER and M. A. SIMON, J. Infect. Dis. **44**:62, 1929.

Four strains of *Brucella melitensis* and *abortus* of different origins were studied. Their acid agglutination optimums were found to be identical, a fact which is believed to be of interest in the consideration of their close relationship, if not their identity.

AUTHORS' SUMMARY.

HEMOLYSIN AND HEMAGGLUTININ FOR SHEEP CORPUSCLES IN HUMAN SERUMS OF ALL ISOAGGLUTINATIVE GROUPS. ETHEL B. PERRY and G. BERNICE RHODES, J. Infect. Dis. **44**:65, 1929.

Hemolysin for sheep corpuscles is present in human serums of all iso-agglutinative groups, though not in all serums of any group, while the heterophilic hemagglutinin is present in nearly all human serums. The dilutions of serum in which hemagglutinin is best demonstrated, 1:1 to 1:3, are often inhibitory for hemolysis. Of 143 serums, 80 were hemolytic for sheep corpuscles in dilutions ranging chiefly from 1:12 to 1:192 with the limiting titer most frequently 1:48. The titers of the lytic A serums were not lower than those of other groups. The hemolysin is independent of the iso-agglutinin and is absorbable by the kidney or liver of the guinea-pig.

AUTHORS' SUMMARY.

HEMOLYSIN FOR SHEEP CORPUSCLES IN PRECIPITIN SERUMS FROM RABBITS. LUDVIG HEKTOEN and ETHEL B. PERRY, J. Infect. Dis. **44**:68, 1929.

Precipitin serums from rabbits immunized with egg white, cow's milk, blood (beef, chicken, dog), sheep serum, swine euglobulin, human pseudoglobulin, and hemoglobins, fibrinogens and thyroglobulins of many different species contain hemolysin for sheep corpuscles. Tests repeated after absorption of the serums with the homologous antigen and with sheep corpuscles indicate that the hemolysin is independent of the precipitin. The possibility of traces of lipoid (also of other substances) must be considered since in the separation of serum proteins the lipoids are known to be precipitated with the globulins.

AUTHORS' SUMMARY.

THE RELATIONSHIP BETWEEN THE HAEMOLYTIC COMPLEMENT AND THE OPSONIC POWER OF GUINEA-PIG SERUM. JOHN GORDON, HUGH ROBINSON WHITEHEAD and ARTHUR WORMALL, J. Path. & Bact. **32**:57, 1929.

Destruction of the hemolytic complement activity of normal guinea-pig serum by small amounts of ammonia does not reduce to any significant extent the opsonic power of the serum for *Staphylococcus aureus*. Incubation of the normal serum at 37 C. for two hours with this organism, in amounts sufficient to remove the opsonic and hemolytic complement activities, does not remove the fourth component of complement. The conclusion is drawn from this evidence that one component of complement at least, the fourth component, is not essential for the opsonic action of the normal serum.

AUTHORS' SUMMARY.

AGGLUTINATION OF RECURRENT FEVER SPIROCHAETES. HELENE SPARROW, UGO LUMBROSO and MARIO LAPIDARI, Arch. de l'Inst. Pasteur de Tunis **17**:279, 1928.

No difficulty was encountered in demonstrating specific agglutination of various strains of spirochetes classified by Dr. Nicolle and his co-workers in the basis of pathogenicity and of immunity. The serum used was secured from animals which had recovered from an infection, sometimes diluted as much as one hundred times with rabbit serum. The spirochete suspension consisted of citrated blood containing a large number of fresh organisms. Incubation of a mixture of the two for half an hour or an hour was followed by examination under the ultramicroscope. Prolonged incubation may result in the lysis of spirochetes, but correct incubation results in a roset-like arrangement often around blood cells or platelets without necessarily loss of motility.

M. S. MARSHALL.

EOSINOPHILIA OF THE BLOOD AFTER INJECTION OF FOREIGN PROTEINS AND IN HEMOCLASTIC SHOCK. V. SPOUJITCH, J. de physiol. et de path. gén. **26**:655, 1928.

Spoujitch injected foreign proteins into several kinds of animals and found that there was considerable difference in the eosinophilic response of the various species. During different phases of anaphylactic shock, produced by subcutaneous or intraperitoneal injection, the eosinophils and neutrophils responded as one group. After shock in man and in most animals there is no eosinophilia, though in guinea-pigs eosinophilia is pronounced.

THE BACTERICIDAL PROPERTY OF CEREBROSPINAL FLUID AS WELL AS INFLAMMATORY AND NONINFLAMMATORY EFFUSIONS OF THE CHEST AND ABDOMEN. P. VON GARA, Klin. Wchnschr. **7**:2386, 1928.

Many of the spinal fluids examined had no bactericidal properties for gram-positive micro-organisms (anthrax, staphylococci). The spinal fluids from two idiots, however, were markedly bactericidal. A relation between this bactericidal property and the Wassermann or Sachs-Georgi reactions could not be established. There were no differences when the material was obtained from patients with or without inflammatory diseases of the meninges. Inflammatory pleural and peritoneal effusions with high specific gravity were markedly bactericidal. This property was fully active even after eight days. Transudates from the chest and abdomen and the noninflammatory fluid of an ovarian cyst were not bactericidal.

EDWIN F. HIRSCH.

SKIN REACTION WITH THE APPLICATION OF DIFFERENT RHUS SPECIES. H. BIBERSTEIN, Klin. Wchnschr. **8**:99, 1929.

Sensitivity, sensitization and desensitization experiments were tried with six different varieties of *Rhus*. The individual *Rhus* varieties of the same season stimulate with varying frequency, and the irritability of the same variety in different years is not the same. The number of persons reacting increased with the increase in the number of *Rhus* varieties. With sensitization and desensitization the varieties and seasons vary (as in I). Sensitization develops with varying rapidity and in a varying percentage of cases. Certain varieties during a given year sensitize 100 per cent. Primarily susceptible persons could not be sensitized toward other *Rhus* varieties more than those naturally insusceptible. The favoring influence of trauma was confirmed. Sensitization sometimes occurred in waves. Desensitization was accomplished as a rule even in those primarily hypersensitive.

AUTHOR'S SUMMARY.

THE GROUP SPECIFIC DIFFERENTIATION OF HUMAN TISSUES. O. M. SEMZOWA and A. A. TERECHOWA, Klin. Wchnschr. **8**:206, 1929.

Human embryos are undifferentiated up to six months so far as antigenic group properties are concerned. The antigenic group differentiation of human

embryos begins at six and one-half months. The group antigens of differentiated embryos are held in the fixed organ cells (liver, kidney, spleen, brain) and in the erythrocytes. Group antibodies of human embryos were at times not demonstrable even in the last months of intra-uterine life.

AUTHOR'S SUMMARY.

PECULIAR SKIN REACTION CAUSED BY HUMAN BLOOD. R. RÖSSLE, München. med. Wchnschr. **75**:1789, 1928.

For a number of years Rössle observed at necropsies that the contact of cadaver blood with the skin of the flexor service of his forearm caused red spots to appear in from twenty to thirty minutes. These spots disappear in from one half to three quarters of an hour. The degree of the reaction varies with the amount of blood; the contact of a large quantity of blood leaves a red ring surrounding a white blanched center. This phenomenon may sometimes be produced even with blood from living and closely related persons. Its cause is not only mechanical but also chemical: substances quickly produced during the escape of the blood irritate the skin.

EDWIN F. HIRSCH.

INFECTION AND IMMUNITY OF THE SKIN IN ANTHRAX. T. BAUTZ and C. AMIRASLANOW, Ztschr. f. Immunitätsforsch. u. exper. Therap. **56**:1, 1928.

The cutaneous method of vaccination by Besredka is by far superior to the subcutaneous one. There is no elective susceptibility of the skin to anthrax as asserted by Besredka.

W. C. HUEPER.

TUBERCULIN AND FIBROBLASTS. ALBERT FISCHER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **56**:24, 1928.

Tissue cells of tuberculous persons react in vitro after addition of tuberculin with no morphologic changes, but with an increased tendency to grow at certain concentrations of the tuberculin. Strains of fibroblasts cultured over a longer period in a medium containing small amounts of tuberculin acquire a definite resistance against tuberculin in large doses. Strains treated with tuberculin react on the addition of tuberculin or bouillon in certain concentrations with increased proliferation.

W. C. HUEPER.

THE INHIBITION OF BACTERIAL GROWTH IN HUMAN SERUM. WOLFF, Ztschr. f. Immunitätsforsch. u. exper. Therap. **56**:279, 1928.

The inhibition of the growth of streptococci for the first five hours, if grown in blood serum exposed to the air for some time, depends on the strong alkaline reaction (p_H 8) of the serum, to which the cocci have to adapt themselves. The growth of *Bacillus coli, Bacillus typhosus* and *Bacillus anthracis* is not inhibited under the same condition. Rabbit serum does not possess this inhibitory quality. The process is regarded as important for the healing of wounds.

W. C. HUEPER.

THE ACTIVE IMMUNIZATION OF CHILDREN AGAINST SCARLET FEVER. S. KORSCHUN and H. SPIRINA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **56**:288, 1928.

For the immunization of children against scarlet fever the authors recommend three series of injections of a vaccine containing, in 1 cc., .1 billion of streptococci killed by formaldehyde and 2,000 skin units of toxin, followed each time by an injection of toxin. The toxin had to be free from protein substances. The frequency of scarlet fever dropped after the introduction of this method from 10 to 16.7 times and fatal cases disappeared almost completely. The Dick reaction became negative in 89.8 per cent of cases. The injection of too high amounts of vaccine may result in the production of a typical scarlet fever syndrome. Increase in temperature and local reaction are usually the only symptoms following injec-

tion. The immunity produced lasts in general more than a year. Vaccination does not protect against the common complication of scarlet fever. Vaccination is contraindicated in nephritis, incompensated heart lesion and acute infectious diseases. The toxin used for the Dick reaction must be freed from proteins to avoid a pseudoreaction. A positive Dick reaction indicates an increased susceptibility to scarlet fever. W. C. HUEPER.

THE INFLUENCE OF CHOLESTEROL ON EXPERIMENTAL ANAPHYLAXIS. L. SURANYI and L. JARNO, Ztschr. f. Immunitätsforsch. u. exper. Therap. **56**:303, 1928.

Reinjection with cholesterinized serum does not prevent anaphylaxis. Animals in which the blood cholesterol is increased by cholesterol feeding before reinjection succumb without exception to anaphylactic shock. Blood with an increased cholesterol content contains a decreased amount of complement. An interrelation exists between the decrease of complement in anaphylaxis and in cholesteremia and the anaphylactic disposition in cholesteremia. W. C. HUEPER.

THE NATURE OF IMMUNITY IN RELAPSING FEVER. I. KRITSCHEWSKI and S. SCHAPIRO, Ztschr. f. Immunitätsforsch. u. exper. Therap. **56**:308, 1928.

The destruction of the spirochetes and the recovery with the maximal amount of immunity bodies is observed in animals with a well preserved reticulo-endothelial system. Rats in which the spleen is removed die from relapsing fever with a complete absence of spirochetolysins. The introduction of antibodies into rats splenectomized or with a blocked reticulo-endothelial system or both at the time of or after infection, acts as a substitute for the lacking function of the reticulo-endothelial system. The mechanism of the defensive function of this system in relapsing fever is represented, therefore, by the secretion of immunity bodies and not by the phagocytic action of the cells. W. C. HUEPER.

THE CONNECTION BETWEEN BLOOD PLATELETS AND SPINDLE-SHAPED CELLS, ACCORDING TO IMMUNITY METHODS. F. T. GRÜNBAUM, Virchows Arch. f. path. Anat. **267**:126, 1928.

In the agglutination of trypanosomes and spirochetes with blood platelets, the other cells of the blood played no part. The platelets of mammals, however, could be replaced in this reaction by the spindle-shaped cells of birds, but not by those of frogs. The platelets of mammals and the spindle cells of birds, therefore, have similar powers. They also have an independent origin, and are not related to the other formed elements of the peripheral blood. Concerning their origin, it can only be said that it is closely connected with the bone marrow; but to which cells of the marrow their development is due, remains a question. The spindle cells of amphibia do not correspond to the third blood elements of mammals and birds. B. R. LOVETT.

IMMUNOLOGICAL STUDIES OF THE CONSTITUENTS OF BACILLUS DYSENTERIAE (SHIGA). K. MATSUMOTO and T. SEKI, Jap. J. Exper. Med. **7**:1, 1928.

The nucleoprotein of *Bacillus dysenteriae* is antigenic in vivo as well as in vitro, whereas the residual substance which reacts specifically with antibacterial serum has no power to induce the formation of antibodies in vivo.

Tumors

MELANOTIC TUMORS. A. HORWITZ, Ann. Surg. **87**:917, 1928.

This article contains a valuable review of the theories advanced concerning the nature of melanotic tumors, chiefly of the skin. The subject of the origin and fate of melanin is also discussed. Horwitz is inclined to accept the theory of the

epidermal origin of these tumors. He states that the lack of pigment in the skin of these tumors is an expression of rapid growth and failure of maturation and differentiation of the cells. His belief is based on the demonstration of malignant changes beginning in the basal cell layer. These tumors are very malignant and treatment is of little avail. More than one half of his cases were cases of tumors of the lower extremities in patients past middle age. N. ENZER.

AN EXPERIMENTAL STUDY OF THE ETIOLOGY OF CHICKEN SARCOMA I (ROUS). W. E. GYE and J. HOWARD MUELLER, J. Exper. Med. **49**:195, 1929.

Acriflavine in itself is a very feeble antiseptic toward the virus of the filtrable fowl sarcoma. Proof of this statement has been obtained by allowing acriflavine to act on filtrates in which cysteine has been dissolved to prevent loss of infectivity by oxidation. Under such circumstances, a very active filtrate cannot be sterilized (in twenty-four hours) with any possible concentration of acriflavine. Feebly active extracts are rendered inert in twenty-four hours by dilution of acriflavine of 1:10,000. In these experiments the acriflavine is neutralized before being added to tumor extracts; otherwise, precipitates are formed which nullify the experiment. When the infectivity of a Rous tumor extract is destroyed by the action of acriflavine in the presence of fresh horse serum, the result is governed by the viricidal action of the serum, acriflavine acting in a merely supplementary way.

AUTHORS' SUMMARY.

HYPERNEPHROMA WITH SUPRARENAL APLASIA. CLAIRE DEBARGE, J. de physiol. et de path. gén. **26**:639 and 668, 1928.

Debarge discusses the case of a previously healthy man, aged 90, who died apparently of bronchopneumonia. Autopsy disclosed, in addition to the pneumonic observations, complete absence of the right suprarenal gland; there was none on the left at the superior pole, but a small mass was found in the retroperitoneal tissue near the tail of the pancreas. Serial sections of this mass revealed nothing which could be identified as suprarenal tissue but there was considerable tuberculous caseation. The right kidney contained a tumor mass which almost doubled its normal size. It consisted microscopically of fibrous tissue, necrotic material, and typical suprarenal cortical cells. Chromaffin cells could not be identified by staining. The tumor cells showed characteristic malignant changes. The author concludes in his second paper that prolonged life is possible with an infinitesimal number of suprarenal cortical cells, and that a strictly cortical malignant hypernephroma can reproduce exactly the function of normal suprarenal cortical cells.

A CASE OF SARCOMA DEVELOPING AFTER RADIUM TREATMENT OF EPITHELIOMA IN THE TEMPORAL REGION. AAGE WAGNER, Acta radiol. **9**:370, 1928.

A description is given of an epithelioma which during treatment with radium is transformed to a sarcoma. Next, the possible causes of the development of the sarcoma are discussed. These are assumed to be as follows: (1) the origin of the tumor as a carcinosarcoma of which only the carcinoma component was destroyed by treatment; (2) the irritative effect of the carcinoma cells on the connective tissue; (3) the influence of roentgen rays on the connective tissue, and (4) direct metaplasia of the epithelial cells. AUTHOR'S SUMMARY.

THE NATURE OF THE ENTITY TRANSMITTING CHICKEN SARCOMA AS EVIDENCED BY EXPERIMENTS ON DESICCATED SARCOMA TISSUE. W. NAKAHARA, Jap. J. Exper. Med. **7**:101, 1928.

The power of the dry tissue of the Rous sarcoma to transmit sarcoma in chickens is seriously impaired by grinding in mortar. The improbability that a chemical substance can be damaged greatly by grinding is emphasized, and it is concluded that the transmission of chicken sarcoma depends on a foreign body, probably the sarcoma cell itself.

CYTOCHROME IN TUMOR TISSUES. H. YAOI, H. TAMIYA and W. NAKAHARA, Jap. J. Exper. Med. **7**:109, 1928.

Cytochrome, the intracellular respiratory pigment discovered by Keilin, was found in large amounts in Fujinawa rat sarcoma and in smaller amounts in Flexner-Jobling rat carcinoma and Bashford mouse carcinoma; Rous chicken sarcoma contained little or no pigment.

Medicolegal Pathology

PUNCH DRUNK. H. S. MARTLAND, J. A. M. A. **91**:1103, 1928.

This sequence of repeated blows on the head occurs in prize fighters and is marked by staggering, mental confusion, dragging of one foot, a tilted head and parkinsonism. It may be the beginning of mental disease requiring subsequent confinement. It is said to result from small hemorrhages in the cerebrum, which are really contusions, and from the alterations to which they lead. Martland found such hemorrhages in 9 of 309 brains of persons with injured heads, none, however, with broken cranial bones. Fractures of these bones are said to interfere with the production of these effects of concussion, presumably by release of pressure which is necessary for tearing by fluids forced by the blows into places too small to contain them. The lesions are chiefly in the basal ganglions.

E. R. LE COUNT.

THALLIUM POISONING. J. H. T. DAVIES and M. C. ANDREWS, Brit. M. J. **2**:1139, 1927.

Two sisters, aged 11 and 8 years, respectively, were given thallium acetate, 8.5 mg. per kilogram of body weight to one and 8.75 mg. to the other. One became severely poisoned with pains in the knees on the twelfth day, four days later the lower extremities became hot, swollen and tender, while on the eighteenth day there was fluid in the knee joints and the patient had convulsions. She was discharged after ten days of treatment in a hospital, but the knees and legs were still tender on the thirty-fifth day. The scalp of each girl became completely hairless for a time.

EXPERIMENTAL STAB WOUNDS. K. FUJIWARA, Deutsche Ztschr. f. d. ges. gerichtl. Med. **12**:65, 1928.

When the features of stab wounds are hidden by drying and clotted blood, they may be fully restored even as long as forty-eight hours after they are made, by washing them with warm water. The size and other peculiarities of stab wounds of the skin are considerably modified by the degree of tension or relaxation of the skin when the wound is made. Stab wounds of the skin and of viscera differ somewhat although made with the same weapon. In parenchymatous organs they do not gap as much, and the end of the wound corresponding to the back of weapons with only one cutting edge are wider than the knife back is thick; in the skin they may be narrower. That the wounds have been made with a weapon with only one cutting edge is usually easily determined by this greater width of the wound at one end. But this may be difficult to decide when the blade is thin. The shape of the wound at the wide end generally matches that of the knife back and may be square or have several angles. When the back of the blade is flat, one of the two corners of the wide end of the wound is usually indented more than the other in the surface of the skin. Tension of the wound end to end often helps to explain the shape of the weapon. When the wound gaps, its length is less than the width of the blade used, but straightening it so as to lay the edges together before measurements are made may yield a dimension greater than that of the width of the knife or sword employed. These are some of the more important conclusions Fujiwara made by a study of wounds of the shaven skin of anesthetized dogs. He used altogether sixteen different weapons — pocket knives, daggers, stilettos, household and butcher knives, knives used in several industries and army weapons.

E. R. LE COUNT.

OCCUPATIONAL AMYOTROPHIC LATERAL SCLEROSIS. M. GÜNTHER and P. HOCH, Deutsche Ztschr. f. d. ges. gerichtl. Med. **12**:68, 1928.

This disease is said to result from strains, severe trauma of the central nervous system, toxemias of different sorts, exposure to cold and other exciting causes in persons predisposed by heredity. The lesions of the cord were formerly regarded as altogether made up of retrogressive changes, but considerable evidence of inflammation has been found by recent studies. The suggestion has been made that the disease may sometimes have an infectious or toxic origin and extend to the nervous system by lymph channels. With the advent of such explanations for its etiology, the relation of amyotrophic lateral sclerosis to occupational disorders and hazards has assumed importance. After reviewing all the circumstances it was decided that two soldiers of the World War were entitled to compensation for having this disease; that in one of them it followed getting wet and severely chilled, and in the other, acute rheumatism due to exposure. A third patient with amyotrophic lateral sclerosis was denied compensation because there were no grounds for supposing it was related to a primary tuberculosis of the eyes.

E. R. LE COUNT.

ALCOHOLISM IN THE TYROL. P. VOGLER, Ztschr. f. d. ges. Neurol. u. Psychiat. **111**:661, 1927.

From many of the institutes of psychiatry of the universities of Austria and Germany there have appeared during the past few years reports of the way the World War affected the numbers of patients with chronic alcoholism, delirium tremens, alcoholic psychosis and other late effects of the use of alcoholic beverages. Similar studies have also been made in Switzerland and Denmark. To these, Vogler adds a statement of what has been noted in that part of western Austria which lies directly east of Switzerland and north of Italy, a portion of Europe not previously embraced. He found a remarkable correspondence between the condition met with there at Innsbruck and those noted in other parts of Europe. He mentions fourteen writers who have made similar studies. Apparently a large part of middle Europe has now been gone over in these investigations.

In all these countries there was a marked subsidence of these manifestations of chronic alcohol poisoning. This began with, and continued throughout, the war. During the reconstruction period there was a gradual return to prewar conditions, and since then the consumption of alcohol has steadily increased so that now it considerably surpasses anything heretofore known, judging by the numbers treated for its effects in these institutes. The review by Vogler comprises a report of 1,857 cases in which the patients were cared for during twenty-three years, ten before and eight since the war. The fall and rise in the number of patients treated, in percentage of the total number cared for and the numbers of men and of women treated all matched one another closely.

E R. LE COUNT.

Technical

THE HINTON GLYCEROL-CHOLESTEROL PRECIPITATION REACTION IN SYPHILIS. C. MORTON SMITH, Arch. Dermat. & Syph. **19**:439, 1929.

As a rule, the Hinton reaction gives a smaller number of so-called "doubtful" readings. It is claimed also that the reaction becomes positive earlier than does the Wassermann and at least as soon as the Kahn reaction. The incidence of false positive reactions is reduced. There are few conditions, thus far recognized, that give false positive reactions. Another point in favor of the Hinton test is that it is possible to work with anticomplementary and somewhat hemolyzed blood. This fact is often of great value to the clinician. It requires far less training to be able to read results of the Hinton test than those of either the Kahn or the Wassermann test. It is applicable to spinal fluid as well as to blood serum. It is apparently more nearly a specific test for syphilis than the others.

AUTHOR'S SUMMARY.

AN IMPROVED TECHNIQUE FOR THE COMPARISON OF ANTISEPTICS BY YEAST FERMENTATION. SARA E. BRANHAM, J. Infect. Dis. **44**:142, 1929.

A simple device for quantitatively collecting the carbon dioxide produced by fermenting yeasts has been made. By this means, the principles of the method of comparing antiseptics by fermentation of yeast have been confirmed and shown to have a general application and the whole procedure has been put on a more dependable basis. This technic not only affords a simple and effective means of comparing the action of many types of antiseptics, but also offers an opportunity for the study of the phases of the process under observation. The simplicity of this method, as compared with the elaborate and laborious procedures employed in many laboratories, commends it for general use.

AUTHOR'S SUMMARY.

ON PHOTOGRAPHING GROSS PATHOLOGICAL SPECIMENS. W. G. MACCALLUM, Bull. Johns Hopkins Hosp. **44**:207, 1929.

Specimens fixed by Kaiserling's method are photographed under water in a tank covered with plate glass. Details must be studied in the original.

A RADIOPAQUE BISMUTH SUSPENSION FOR ANATOMICAL, HISTOLOGICAL AND PATHOLOGICAL RESEARCH. EBEN C. HILL, Bull. Johns Hopkins Hosp. **44**:248, 1929.

A method using bismuth oxychloride suspended in a solution of gum acacia has been found to be most satisfactory for postmortem roentgen study of the vascular systems. For most purposes a suspension of 20 per cent bismuth oxychloride in 11 per cent solution of acacia gives excellent delineation, but for specific tissues the percentages of both the bismuth and the acacia must be varied to secure optimal results. The acacia serves as an agent to aggregate the finely divided particles of bismuth, and by employing cloth filters of various meshes, unit masses of any desired size may be obtained. Tissue that has undergone an injection of this radiopaque suspension may be studied microscopically either as cleared specimens or in stained histologic sections; in both, the bismuth-filled area is clearly delimited from the surrounding tissues.

AUTHOR'S SUMMARY.

THE SEDIMENTATION RATE OF ERYTHROCYTES. H. B. NEWHAM and P. H. MARTIN, Quart. J. Med. **22**:145, 1928.

The present study was conducted in twenty-five patients suffering from sprue, kala-azar, malaria, secondary anemia, trypanosomiasis, cirrhosis, dysentery and amebic hepatitis. Liver function was determined by the levulose test. No change in liver function, however, was found which would parallel the changes in the rates of sedimentation. Differences in specific gravity of the erythrocytes apparently had no bearing on the phenomenon. The same is true of the viscosity of the plasma and of the size of the erythrocytes. One positive fact appeared, namely, that most of the rapidly sedimenting bloods showed auto-agglutination of the erythrocytes.

N. ENZER.

HEMATOXYLIN AS A REAGENT FOR IRON. M. MÜHLMANN, Virchows Arch. f. path. Anat. **266**:697, 1928.

Interested in the small quantities of iron in the central nervous system that do not give the ordinary reactions for this metal, the author investigated the possibilities of hematoxylin as an iron reagent. He concluded that staining with hematoxylin depends on an alkaline reaction and the presence of iron, usually suboxides. Two sorts of iron-containing substances were found, one in myelin sheaths, which resisted the action of acids but disappeared after treatment with lipoid solvents, and the other in the pigment of the substantis nigra and in the capillary endothelium, resistant to both reagents. He suggested phagocyted red cells as the source of the iron in the latter.

B. R. LOVETT.

Society Transactions

NEW YORK PATHOLOGICAL SOCIETY

Regular Meeting, Feb. 13, 1929

HARRISON S. MARTLAND, M.D., *Presiding*

VIRULENT ANTHRAX BACILLUS FROM A CASE OF MALIGNANT PUSTULE IN MAN.

ADELE E. SHEPLAR and MARJORIE B. PATTERSON.

On Dec. 31, 1928, P. W., a man, appeared at the outpatient department of the New York Post-Graduate Hospital with a lesion on the right side of the neck of five days' duration. He was sent to the laboratory for bacteriologic examination. The lesion presented a very dark crater-iike center, approximately 15 mm. in diameter, surrounded by confluent vesicles containing a pink-colored thin fluid, the total area approximating 50 mm. in diameter. Microscopic examination of the fluid obtained from the vesicles revealed large numbers of spherical organisms evidently staphylococci and a few large rods. Plate cultures showed large numbers of colonies of staphylococci and a smaller number of typical Medusa head colonies of the anthrax bacillus.

On intraperitoneal inoculation into a mouse, a saline suspension of the colonies killed the animal within fifteen hours, and on subcutaneous inoculation into a guinea-pig produced death in thirty-six hours. A small fragment of the spleen of the guinea-pig was placed in a subcutaneous pocket of a rabbit and this animal died after five days with extensive subcutaneous edema about the point of inoculation. A secondary infection was present, however, and necropsy disclosed a complicating old pericarditis, possibly due to previous use of the animal for bleeding by heart puncture. A second rabbit was inoculated by placing a loopful of a twenty-four hour agar slant culture of the third cultural generation of this strain into a subcutaneous pocket. This animal died after sixty hours. Subcutaneous hemorrhagic edema was slight and limited to the region of the inoculation, an area approximately 40 by 50 mm. The anthrax bacilli were found distributed through all the organs and present in the heart blood.

The patient was employed as a chauffer by a furniture dealer and part of his time was used in furniture repair and upholstery work. It is probable that he had come into contact with animal hair. He said that the lesion had begun as a small pimple at the side of the neck some five days before. After appropriate serum treatment, the patient made a good recovery.

The observation is of interest because the lesion here corresponds to the classic malignant pustule and contains anthrax bacilli of full virulence similar to the Pasteur virus strain capable of killing rabbits. The nature of the animal hair concerned in the present instance is unknown.

RESORCINOL TESTS IN RELATION TO DISEASES WITHOUT CONCOMITANT TUBERCULOSIS.

ADELAIDE B. BAYLIS.

A report of 163 tests on 156 patients with various diseases, without recognized concomitant tuberculosis, tested by resorcinol flocculation, reveals the fact that certain diseases other than tuberculosis are capable of giving false reactions. It is, therefore, concluded that when these diseases exist without apparent concomitant tuberculosis, diagnostic observations should be confirmed by further laboratory and clinical examinations. A comparison of the Vernes and Baylis tests shows a close correlation between the two methods, and while they are useful in differential diagnosis, their chief value lies in their ability to measure the degree of activity in recognized tuberculosis.

DISCUSSION

WARD J. MACNEAL: I should like to emphasize a point which Miss Baylis made at the end, in order that it may be clearly understood, because certain recent visitors to our laboratory appear not to have appreciated this point which Miss Baylis has attempted to emphasize in all the publications on this subject. Tuberculosis is not diagnosed satisfactorily by this test; we make the diagnosis of infection with the tubercle bacillus by entirely different means. This test, moreover, makes no pretense to measure the extent of the lesion in the body; however, it does appear to indicate the degree of activity of the process. Any one who is familiar with the pathology of tuberculosis will realize that we need this sort of a test. We already possess many means of diagnosing the presence of the tubercle bacillus in the body with a fair degree of accuracy and of measuring the amount of tissue involved. This resorcinol test does not purpose to do those things; it is used to indicate the state of activity of the tuberculous process.

FACTORS INFLUENCING MOTILITY OF SPERMATOZOA. J. A. KILLIAN, J. F. MCCARTHY, C. T. STEPITA and M. B. JOHNSTON.

Observations have been made on the influence of variations in viscosity, osmotic pressure and H-ion concentration on the motility of spermatozoa in semen.

Increasing viscosity of semen by the addition of gelatin solution was followed by a proportionate decrease is motility. Nonmotile spermatozoa in viscid specimens could be rendered motile by the addition of equal volumes of 0.5 molar dextrose.

A depression of the motility of spermatozoa followed the addition of aqueous solutions of nonelectrolytes to the semen. Molar solutions destroyed motility entirely. Five-tenths molar solutions depressed the motility to about one half the control. Urea, however, was a notable exception to this general rule.

Aqueous solutions of two ion salts depressed motility to a greater extent than nonelectrolytes; aqueous solutions of three ion salts diminished the motility below that observed for two ion salts.

Variations in H-ion concentration, between p_H 5 and p_H 8.3 did not influence the motility of spermatozoa, but increasing the H-ion concentration or decreasing it beyond these limits was followed by a depression of the motility.

DISCUSSION

JACOB M. RAVID: I wonder whether one can draw any analogy from the motility of *B. coli*—*B. typhosus* groups or of any other motile bacteria to that of the spermatozoa. We know very well what an important factor temperature plays in the motility of micro-organisms. I should therefore like to ask the authors what their experience was in regard to this point and also whether they paid special attention to the temperature in all their experiments.

C. T. STEPITA: This study of the factors influencing the motility of the spermatozoa of the semen, credit for which is in great part due to Dr. Killian, was stimulated by a study by Drs. McCarthy, Ritter and Klemperer, made at the laboratories of the New York Post-Graduate Hospital, relative to the anatomy and the histology of the verumontanum and the ejaculatory ducts. At this time it was demonstrated that catheterization of the ejaculatory ducts was feasible. By means of such catheterization the seminal vesicle, a hitherto obscure organ, is brought into the searching spotlight of orderly scientific scrutiny, and by this means not only the physical conformation but the unmixed bacteriology of the vesicles, as well as the viability of the spermatozoa in their natural habitat, may be determined. One has also the means of observing the reaction of the seminal vesicular secretions uncontaminated by the prostatic secretions and, finally, in the presence of sluggishly motile or dead spermatozoa it may be possible by direct treatment to remove the conditions inimical to their viability.

The present study was not concerned so much with the highly technical work of catheterization. We were more interested in the actual study of the specimens of semen. Dr. Killian made a study a number of years ago of the

biochemistry of the prostatovesicular secretions, during which the most interesting phenomenon of glycolysis, or sugar or carbohydrate utilization by spermatozoa in the semen, was demonstrated, and it was then determined that spermatozoa in vitro, which after six hours had become sluggishly motile, could be markedly activated by the addition of 5 per cent dextrose solution. By a play of the imagination, one can readily see how, in an individual with sluggishly motile spermatozoa as a factor in incapacity for procreation, such spermatozoa might be revivified in vivo. These studies, then, are a continuation of the study of glycolysis made some time ago. As Dr. Killian has explained, the three important factors influencing the motility of spermatozoa are viscosity, osmotic pressure and reaction. The viscosity or plasticity — there being a fine distinction between the two terms — may be an important factor, and the motility of the spermatozoa may be restored to the maximum by the addition of equal volumes of 4.5 per cent dextrose, buffered with phosphates to p_H 7.6. The reaction is probably least important because between p_H 5 and p_H 8.3 the motility remains unchanged and, during the study of glycolysis, it was observed that the reaction of the semen was p_H 7.6 during the first six hours, despite the fact that there was an increase in lactic acid above 100 per cent of the control, there evidently being an efficient and effective buffer mechanism in the semen itself. These studies offer a fertile field for future investigation. A practical application has been made in only one instance. A couple, married eight years and anxious to have children, came to see Dr. Killian. He advised them of the proper solutions to use and the proper strengths to employ, and the wife became pregnant.

J. A. KILLIAN: Dr. Ravid raised the question of the influence of temperature on motility. From our own observations, we can definitely say that variations between 15 and 40 C. have no demonstrable effect on the motility of spermatozoa. All these experiments were carried out between 20 and 25 C., so that we can exclude temperature as a factor influencing the motility. The only experiment which varied from that was one consisting in the addition of gelatin and the increase of viscosity by lowering the temperature.

THE HALOGEN BALANCE IN BROMIDE THERAPY OF EPILEPSY. J. A. KILLIAN, J. NOTKIN, T. GARCIA and L. HALPERN.

In this country, the use of bromides as antispasmodics has been discredited owing to the appearance of ominous symptoms and complications following their indiscriminate administration.

In order to secure the maximum antispasmodic effect and to obviate complications, the administration of bromides should be guided by a knowledge of their fate in the human organism and their influence on the components of normal body fluids.

In the study of the effect of ingested bromides in the treatment for epilepsy on the female service of the Manhattan State Hospital, we have determined the distribution of the halides between the blood plasma and the erythrocytes.

After the continuous administration of halides, the total halides are increased above the normal with a replacement of a portion of the chlorides by bromides. This does not influence the distribution of chloride between the blood cells and the plasma, and the bromide is distributed between blood cells and plasma in a manner similar to that of the chlorides.

Bromide is excreted in the urine with chloride in the same proportion in which these halides are found in blood. The bromine ion apparently does not diffuse into spinal fluid as readily as the chlorine ion. The blood plasma of epileptic persons on bromide therapy shows an opalescence which is not associated with any change in the lipins of the blood or the serum proteins of the blood.

DISCUSSION

J. NOTKIN: Attention is called to the work of von Wiss, Frey, Ulrich and Bernoulli, particularly the latter, who has done considerable experimental work

with bromide in animals and men, and who finally evolved the conception of the "relative bromide content," which consists in a substitution of only a certain amount of sodium chloride by sodium bromide, and is expressed in a percentage rate. According to Bernoulli, the relative bromide content should not be higher than 20 in order to avoid the complications of bromide intoxication. In my experience, I have found that the relative bromide content may be much higher and the signs of complication are not always due to bromide intoxication but to lack of sodium chloride in the organism. I believe that what really matters is the necessary physiologic minimum of sodium chloride in the organism, which apparently varies from one person to the other, and from one race to the other. The Wuth's calorimetric method of bromide estimation in the blood is discussed, and here again I found very much higher figures. I shall show a comparative study of phenobarbital and bromide therapy of a large number of epileptic patients treated at the Manhattan State Hospital since July, 1926.

J. A. KILLIAN: The undesirable effects of bromide therapy might be attributed to one of two conditions, either an accumulation of the bromide ion in the body tissues or fluid, or a loss of chloride. When it comes to the question of following these cases from the clinical standpoint, from the results shown on the chart it is evident that analysis of the urine is adequate because it is shown that the bromide is excreted in the urine in the same proportion in which it is in the blood. Consequently, from the urinalysis one can ascertain the relative concentrations of bromide and chloride in the blood.

THE SO-CALLED "LIPOID NEPHROSIS." NICOLAS M. ALTER.

Is "lipoid nephrosis" a disease entity with a pathologic process of the kidney that is responsible for the characteristic clinical picture? This is the obtruding question to any one familiar with this disease.

Clinical, but particularly pathologic data are still lacking and are much needed to attempt to answer this and many other questions that turn up during the study of this condition.

A white boy, aged 8 years, was admitted to Post-Graduate Medical School and Hospital on Aug. 10, 1928, with edema of the forehead and legs and "urinary disease"—albuminuria and casts. On admission, the patient showed edema of the face and ankles. The blood pressure and temperature were normal. The chemical analysis of the blood showed: urea nitrogen, 12.8; cholesterol, 0.357; albumin, 1.75, and globulin, 1.58. The blood count revealed: red blood cells, 4,192,000; white blood cells, 15,200, and polymorphonuclears, 72 per cent. The present illness consisted in an aggravation of symptoms for the last three to four weeks, since a cold, but was practically continuous for from two to three years.

He had frequent colds and sore throat, whooping cough and infection of the ear in 1928. On Aug. 22, 1928, the patient became acutely ill, with a temperature of 101 F., and convulsions. He died with symptoms of uremia on August 24. On August 22, the blood pressure was 108 systolic and 68 diastolic; the blood urea nitrogen was 25.8. On August 23, the blood pressure was 124 systolic and 100 diastolic; the blood urea nitrogen, 53.7. Autopsy was performed on Aug. 24, 1928.

The body weight was about 30 Kg. General edema was present. The heart weight was 145 Gm. The thickness of the left ventricle was 7 mm. The weight of both kidneys was 375 Gm. Cross-section showed a uniformly thick cortex (13 mm.) of a grayish, translucent, edematous appearance. There was a yellow, somewhat opaque streaking. The papillae were dark. The renal pelvis was narrow. The liver weighed 765 Gm. The capsule was smooth. On cross-section, the parenchyma was pale brown throughout. Scattered uniformly small specks of yellow areas were seen. The suprarenal glands together weighed 8 Gm. The layers were thin and pale. The retroperitoneal lymph nodes measured from 2 to 3 cm. in diameter; they were soft. The lungs were edematous.

Microscopic sections of the kidneys showed a striking picture. The streaks in the gross specimen corresponded to various stages of a progressive pathologic

process. At an early stage in the stroma, there were accumulations of pale cells which were larger than the tubular cells and had small dark nuclei; the glomeruli within such areas may show no lesion or early deposit of hyaline material within the tuft. The large pale cells stained intensely with fat stains. In frozen sections, the fat was double refractile. The glomerular epithelium contained much less fat. The lipoidal cells were in the interstitial tissue. In the older areas, these cells were replaced by fibroblastic proliferation and lymphocytes. The tufts of the glomeruli showed gradual filling up with hyaline material, which did not take the amyloid stain. The tubular epithelium showed an uneven distribution of the finer and coarser fat globules with fat stain, mostly in the high epithelial cells of the convoluted tubuli of the first order and ascending portion of Henle's loop. In the lumen of the tubuli casts were seen and many stained intensely with fat stain.

Sections of the liver, suprarenal gland and retroperitoneal lymph node showed also accumulations of lipoidal cells, which were similar to those in the kidneys. In the liver, the lipoidal cells showed transition to hyalinization and necrosis.

From the pathologic observations it was obvious that we were dealing with a general constitutional condition. The striking feature was the accumulation of lipoidal cells in many organs; this suggested a general lipoidosis of the endothelial cells with marked disease of the kidneys.

The clinical picture corresponded to the pathologic observations in the interpretation of the glomerular changes which were secondary to the fatty and hyaline changes not unlike amyloidosis. In many other respects, too, the condition showed some similarity to general amyloidosis.

In this case we seemed to deal with a general lipoidosis, which had produced grave secondary anatomic changes in the kidneys, which then became responsible for the characteristic clinical picture. There was morphologic as well as clinical evidence that the essentially tubular kidney disease of long duration terminated with advanced glomerular injury, causing renal insufficiency and uremia.

DISCUSSION

HARRISON S. MARTLAND: I suppose there is no doubt from the clinical history of this case and the gross and microscopic studies that it is a typical nephrosis, such as has been described by Epstein. The great controversy, however, is how many kidneys should one take away from the parenchymatous group and place in this category.

ACUTE NEPHRITIS IN RATS AFTER INTRAVENOUS INJECTIONS OF COLLOIDAL LEAD. LOUISE H. MEEKER.

The kidneys of albino rats show striking parenchymatous changes after lead poisoning.

Our observations are based on forty-four rats treated with colloidal lead. The lead solution was given intravenously for the most part. In a few instances, subcutaneous and intraperitoneal injections were given. The lead was prepared under the direction of Dr. Sheplar. The method was that of Woodward. The actual amount of lead in each colloidal preparation was determined under the direction of Dr. Killian. Dr. Killian also later analyzed the kidneys of the rats and found lead present.

The duration of the lead treatment in the animals was as follows:

1 for 55 days	1 for 36 days	5 for 19 days	1 for 12 days
2 for 54 days	1 for 30 days	1 for 18 days	1 for 9 days
1 for 52 days	3 for 25 days	1 for 17 days	2 for 8 days
1 for 47 days	2 for 21 days	3 for 14 days	1 for 7 days
4 for 44 days			

The number of injections varied from two to ten; the number of days between the injections varied from three to ten, seven days being the usual interval. Ordinarily, the rats were killed two and three days after the last injection of lead. In five instances, the last injection was seven, sixteen, eighteen, nineteen and twenty-nine days before death. Four of the rats died from lead poisoning.

The total amount of lead given to any one rat varied from 3 to 176 mg. The rat receiving 176 mg. was treated fifty-five days, and the largest amount of lead given at any one time was 26 mg., or twice the lethal dose.

The changes in the kidney were most evident in the second portion of the convoluted tubules (fig. 1, *a* and *b*). The epithelial cells were swollen and the cell boundaries lost very early, with cell débris filling the lumina. The nuclei became swollen and pale with fragments of chromatin forming clumps. Ovoid bodies staining pink with eosin appeared near the nucleoli or by transformation of the nucleoli (fig. 2, 32). As these bodies enlarged, the chromatin granules collected on their surface and the nuclear membrane slowly disappeared. The pink-staining bodies were extruded into the lumen of the tubule and became larger and darker. The outline of these now dark bodies usually showed serrations and lamellation, as many as eight or ten concentric lamellae being counted in many

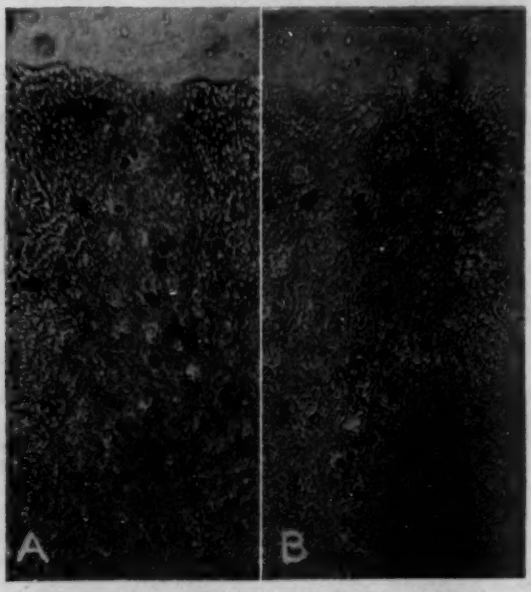

Fig. 1.—The black bodies are in the convoluted tubules. *a*, after exposure to hydrogen sulphide in an acid solution; *b*, unstained. Frozen sections, low power.

instances (fig. 2, 23). Tubules showing the early intranucleolar forms of these bodies occasionally showed mitotic figures in adjacent cells (fig. 2, 25). Where these dark bodies were most numerous the tubule epithelium was entirely disintegrated. The bodies stain black when exposed to hydrogen sulphide in a weakly acid solution, indicating a lead content. They also stain blue by Perl's test for iron.

Bell has emphasized the affinity between lead and lecithin. It is suggested that lead may have united with the lecithin or a similar lipin in the tubule epithelium to form these bodies. To follow the complicated reaction between them would require in vivo studies similar to those of Leathes, MacLean and White, together with much further animal experimentation. Budding forms and minute droplets are seen and are similar in reaction to the larger bodies. These droplets also suggest myelin forms of lipins. The bodies are the only elements in the sections that give a positive lead reaction by microchemical tests.

As a rule, the glomeruli in the affected areas of the kidneys show no change. In some instances, however, the glomeruli show marked congestion and numbers of polymorphonuclear leukocytes and occasionally albuminous exudate in Bowman's space. In other words, the glomerular changes are always acute and transient. It is to this acute glomerular inflammation that we consider the hemoglobin and iron in these bodies is chiefly due. Lead does not unite with hemoglobin.

There are no changes in the collecting tubules, in the interstitial tissue or in the blood vessels. Forty control rats showed no kidney lesions as described for the rats treated with lead.

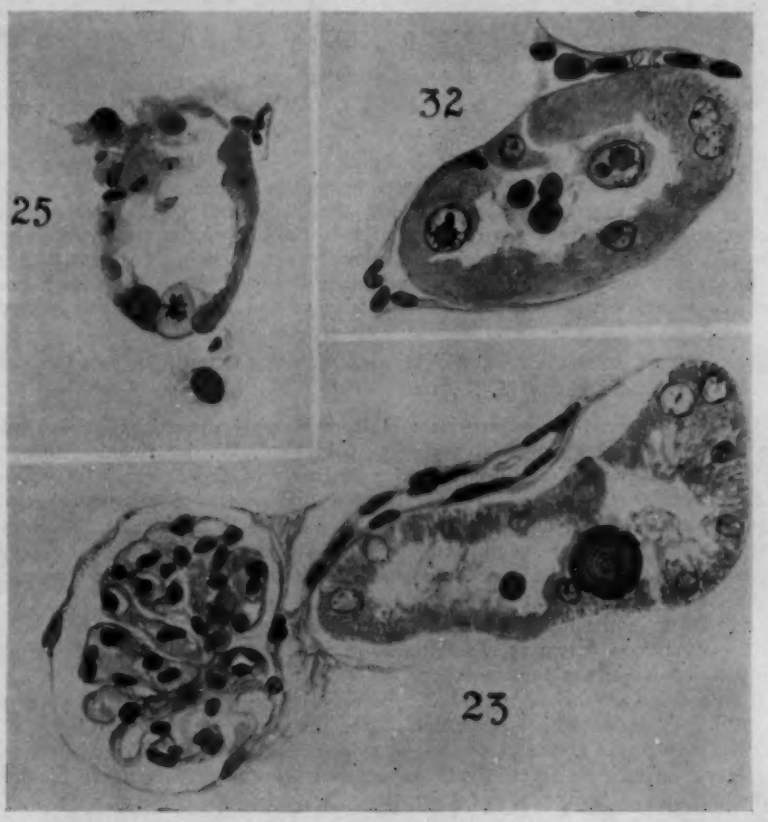

Fig. 2.—Rat 25: The experiment lasted twenty-five days with four intravenous injections. The total amount of lead injected was 3.8 mg. High power; hematoxylin and eosin. Rat 32: The experiment lasted fifty-five days with six intravenous injections. The total amount of lead injected was 111 mg. Eosin-staining bodies in pale nuclei with chromatin at the periphery are present. The black bodies in the lumen of the tubule show eosin-staining bodies in the center. High power; hematoxylin and eosin. Rat 23: The experiment lasted twenty-one days, with four intravenous injections. The total amount of lead injected was 3.5 mg. Black bodies are present in the lumen of the tubule. The larger body shows lamellation and crenations. The glomerulus (left) and blood vessel (above) show no pathologic changes. High power; hematoxylin and eosin.

In summarizing, it may be said: colloidal lead produces a destruction of tubule epithelium of the kidney somewhat similar to the action of other metals, such as mercury. The portion of tubule affected is that concerned in elimination. Recovery may occur after one, two or three insults, but finally the regenerative capacity is destroyed and no further recovery is seen. Individual rats vary greatly in their susceptibility to colloidal lead. Lead may be recovered from the kidneys showing the lesions described.

DISCUSSION

JAMES EWING: Did you find any colloidal particles of lead in the endothelial cells, such as are found in cases in man?

LOUISE H. MEEKER: There were no particles of lead in the endothelial cells of the kidney. The spleen and liver have not been carefully studied by me as yet. My purpose at this time has been to show a definite reaction to lead in the kidney since this has been frequently disputed. We believe that we have a reaction comparable to that of other heavy metals.

HARRISON S. MARTLAND: It is hardly fair to compare the experience I have had with a very stable and apparently nontoxic form of colloidal lead with that of those using colloids approaching the Blair Bell type. Our lead was phagocytosed to an enormous extent by the histiocytes of the reticulo-endothelial system and very little ever reached the malignant growth. In time, however, it was toxic and produced terrific uncontrollable anemias. In human beings and in animals it was very toxic to the kidneys and produced intense nephritis.

ARRANGEMENT OF THE SMALLER ARTERIAL VESSELS IN THE SPLEEN. WARD J. MACNEAL and JACOB M. RAVID.

Human spleens from necropsies and from surgical operations were fixed in the distended state by arterial perfusion (MacNeal, W. J.; Otani, Sadao, and Patterson, M. B.: The Finer Vascular Channels of the Spleen, *Am. J. Path.* **3**:111 [March] 1927). Serial sections at a thickness of 5 microns have then been prepared. For study of the course of the arteries and veins, drawings of every tenth section at a magnification of 20 diameters were made, transferred to wax plates 1 mm. thick, and these plates were put together for reconstruction of the vessels and follicles in relation to them.

For study of the finer arterial vessels pertaining to an individual lobule, each serial section was drawn at a magnification of 200 diameters, and reconstruction was carried on in the same manner.

The models are not yet finished. The reconstruction, however, has progressed to such a point that the architecture of the splenic lobule and the interrelationships of the lobules and blood vessels have been disclosed. As a rule, the arterial supply of each lobule is terminal, but occasionally one may recognize a slender communicating arterial twig coming from an adjacent trabecular artery and joining on to the eccentric arteriole of the lobule. From the eccentric artery there are given off the follicular capillaries, which form an anastomosing meshwork of thin-walled closed capillaries within the follicle and terminate by opening into the intercellular pulp spaces of the marginal zone of the lobule. These vessels are minute, often collapsed in part, and are extremely difficult to trace in serial sections. Larger branches of the order of fine arterioles take origin from branches of the eccentric artery and extend into the marginal zone, where they are surrounded by the ellipsoid sheaths of Schweigger-Seidel. They give off recurrent or centripetal capillaries which curve toward the follicle and terminate by opening out into the intercellular pulp spaces of the marginal zone. Other small branches arising from the same vessels continue in a centrifugal direction as the capillaries of pulp cords, to terminate in the intercellular spaces of the pulp cord at a distance from the follicle.

Regular Meeting, March 14, 1929

HARRISON S. MARTLAND, *President, in the Chair*

MULTIPLE FIBROMATOUS POLYPI OF THE INTESTINE, ASSOCIATED WITH CHRONIC TUBERCULOSIS OF THE ILEUM AND CECUM. DAVID PERLA.

An instance was reported of multiple fibromatous polypi of the intestinal tract in a middle aged man suffering from chronic hyperplastic tuberculosis of the intestinal tract. The polypi were most prominent in the rectum, where they produced partial obstruction because of the large size of the fibromatous masses. A correlation between the chronic tuberculosis of the intestine and the development of these tumors was suggested. To the author's knowledge, this is the second instance on record of multiple fibromatous polypi of the intestinal tract.

DISCUSSION

NICHOLAS ALTER: It is known that tubercle bacilli have a stimulating effect on the surrounding tissue. They produce a specific granulation tissue, but whether it can produce a stimulation to other tissues of a neoplastic nature has not been sufficiently emphasized, although it has been mentioned time and again that there may be some relation between tuberculosis and malignant disease. Adler, in his monographs published sometime ago, showed a close relation between tuberculosis of the lung and carcinoma. In my experience, I often found malignant disease associated with tuberculosis. I remember a case of carcinoma of the esophagus associated with tuberculosis and another of testicular teratoma associated with tuberculosis; there are many other cases. I recall the old theory of Ehrlich that he propounded in connection with his carcinosarcoma in cancer produced in mice. Ehrlich brought forward the explanation that the whole course depends on the nutrition of the tissue and that the various tissues depend on various food supplies. This explanation would somewhat explain why in one case tuberculosis produces a specific granulation tissue and in another, a proliferation of a neoplastic nature. I am thoroughly convinced that tuberculosis and cancer have a close connection.

HARRISON S. MARTLAND: I do not know whether I missed some of Dr. Perla's remarks, but did I understand him to say that multiple fibrous polypi in the colon constituted an unusual or rare lesion?

DAVID PERLA: Yes.

HARRISON S. MARTLAND: In the case presented, did you interpret the polypi as existing before the tuberculous ulcers?

DAVID PERLA: That question would be difficult to answer. I intimated that there might be some relationship between the long-standing chronic hyperplastic tuberculosis of the intestinal tract and the development of these multiple fibromatous polypi. It is well known that in association with chronic irritations of the intestinal tract with strictures, and with hyperplastic tuberculosis, polypi are frequently seen; in strictures produced by neoplastic changes, one sees filiform elongated polypi around the stricture, so there is no doubt that chronic irritation can stimulate the overgrowth of mucous membrane sufficiently to produce these tumor formations. On that basis, I suggested the possibility that the long-standing infection might also have stimulated the peculiar fibromatous processes.

MYASTHENIA GRAVIS WITH MULTIPLE THYMIC GRANULOMAS. NICHOLAS M. ALTER.

The pathologic process of this rare disease is still the subject of a great deal of controversy. Every case that can bring additional information should be recorded. The extensive postmortem observations warrant this report.

A white woman, aged 31, married, was admitted to the New York Post-Graduate Hospital on Sept. 26, 1928, with the chief complaints of ptosis, internal strabismus, dysphagia, dysphonia, dysmasesis, general weakness and loss of weight. The illness began about six months before admission with ptosis of both lids.

Two months before death, she began to have difficulty with speech. For the past ten days, she could not swallow. There was a gradually increasing weakness of the muscles of the back, neck and jaw. On physical examination, the patient was well nourished and had marked ptosis of both eyes, more marked on the left than on the right. She had internal strabismus. There was bilateral paralysis of the face. The patient could not laugh. Speech, swallowing and breathing were greatly impaired. The blood pressure was 130 systolic and 80 diastolic. The spinal fluid was normal.

Autopsy revealed a symmetrical enlargement of the thyroid with a hard nodule over the isthmus. Discrete lymphnodes were palpable on both sides of the neck and in the inguinal regions. The thymus was large, measuring 9 by 4 by 4 cm. On section, a tumor-like mass was seen, with a hard, partly calcified capsule, and a red, pulpy center. The mass measured 6 by 4 by 4 cm. A similar nodule was situated above the isthmus of the thyroid, measuring 25 by 22 by 13 mm. The thyroid was fleshy and firm. On cross-section, the left lobe contained a nodule, 14 by 10 by 10 mm. The heart weighed 220 Gm. The aorta measured 3.5 cm. in circumference. On gross examination, the brain and cord revealed nothing unusual. The uterus and ovaries were infantile.

Microscopic section of the thymus mass showed hyperplasia of thethymic tissue with a few large Hassall bodies. In most areas large, pale, epithelial cells predominated, in others small round cells. There was irregular fibrosis with marked hyalinization, particularly in the capsule. The second nodule above the isthmus of the thyroid was similar morphologically. Sections of the thyroid showed a diffuse epithelial hyperplasia. The colloid secretion was diminished. The stroma was increased and contained diffuse lymphocytic infiltration. The circumscribed nodule consisted essentially of epithelial-like cells. Sections of the heart and the muscles and those of the tongue and the muscles of the neck showed some lymphocytic infiltration and atrophy of the striated fibers.

The pathologic observations indicated extensive constitutional changes. The most conspicuous features were the multiple tumor-like nodules of thethymus and thyroid, the small heart and narrow aorta, general lymphadenopathy and lymphoid infiltration of the muscles of the neck.

DISCUSSION

JAMES EWING: I find it difficult to form any definite conclusion regarding the significance of the lesions in the thymus and the thyroid. Their interpretation would require much more careful study than I am able to make, even from this full report. The suggestion that the whole condition in this case is a phase of status lymphaticus is most interesting. I should like to ask Dr. Alter if he has some computation of the number of cases of myasthenia gravis reported in which there were such definite signs of status lymphaticus. In this case, these signs seem to be extremely well marked.

HARRISON S. MARTLAND: Did the spleen and intestinal tract show a marked increase in lymphoid tissue?

NICHOLAS ALTER: Yes, there was a large amount. With reference to Dr. Ewing's remark, there was not a single definite report emphasizing the presence of status lymphaticus. In some cases the thymus is described as enlarged, but the authors do not refer to the other requirements for status lymphaticus. Neither is there any investigation of the nature of the enlargement of the thymus, except for Bell's work in 1912; this author tried to classify some other cases and his own as benign thymoma. There is no mention of the concept of these nodules as infectious granulomas.

ERYTHROBLASTEMIA OF INFANTS; A STUDY OF SO-CALLED VON JAKSCH'S ANEMIA. B. R. WHITCHER.

Recently, cases of so-called von Jaksch's anemia have come to be regarded as belonging to the regularly accepted groups of secondary anemias in children, or as

leukemias incorrectly diagnosed. A certain type of an anemia, however, in which an unusually large number of nucleated red cells is found, does occur in infants; these red cells sometimes outnumber the leukocytes and constitute an outstanding feature because of which this condition should be classed as a distinct type of anemia of unknown etiology.

Occasionally during the past seven or eight years, an infant born at full term with anemia and showing such an unusually large number of erythroblasts in the blood, has been admitted to the Babies' Wards at the Post-Graduate Hospital; the cases of three such infants were described. The first was that of a boy, aged 9 months, who was a mongolian idiot. The blood picture showed 125 nucleated red cells to 100 leukocytes. The second case was that of a boy, aged 5½ months, with malnutrition, who showed 92 nucleated red cells to 100 leukocytes. The third was that of a boy, aged 6 months, who had malnutrition and disease of the middle ear and showed 110 erythroblasts to 100 leukocytes.

From the study of these cases, the most conspicuous feature seen is the unusually great abundance of immature red cells, far greater than in the usual form of secondary anemia, in which erythroblasts do occur; therefore, it would seem that a more suitable name for this condition would be erythroblastic anemia, or erythroblastemia of infants, instead of pseudoleukemia infantum or von Jaksch's disease.

DISCUSSION

HARRISON S. MARTLAND: I suppose that all these cases would have been diagnosed von Jaksch's anemia.

B. R. WHITCHER: That was the diagnosis made formerly.

HARRISON S. MARTLAND: Although I am opposed to giving new names to diseases of the blood unless their etiology is definitely determined, the designation suggested, "erythroblastemia of infants," is perhaps more descriptive than von Jaksch's anemia.

JAMES EWING: Were any of the nucleated red cells easily confused with lymphocytes?

B. R. WHITCHER: No. On the whole, especially in the first case, the nucleated red cells showed little polychromatophilia. In some the cytoplasm showed a slight lavender color, and in others a slight slate color, but in most of them it showed the same salmon-pink color that the nonnucleated red cells did. In the other cases, the nucleated cells in some instances showed a slight slate color, and some of the cells showed rather ragged edges, but they could readily be distinguished from the lymphocytes.

JAMES EWING: You said that the diagnosis of leukemia was thought of.

B. R. WHITCHER: That diagnosis was thought of, but after making the second count I decided there was no leukemia, and that the condition was von Jaksch's disease.

HARRISON S. MARTLAND: I do not believe that in these cases there is any doubt about the identity of the nucleated red cells. There is usually no difficulty in accurately identifying cells in blood smears. Identification is not so easy, however, in sections of bone marrow in which it is often impossible, even by special stains, to state whether certain cells are of erythroblastic, myeloblastic or even lymphoblastic origin. For this reason, I have long advocated the study of smears of bone marrow in preference to sections for accurate interpretation.

JAMES EWING: I have an idea that some of the so-called low-grade leukemias of infants are of this type, in which the nucleated cells with little or no hemoglobin are mistaken for lymphocytes.

B. F. YOULAND: In the medical service of Flower Hospital, an infant was born who died about five days after delivery. At autopsy, the skin presented a distinctive pale, indefinite, yellow color suggestive of pigmentation and apparently resembling that described by Dr. Whitcher. When the body was opened, the internal tissues and organs presented a somewhat similar discoloration. The

splenic lymph nodes stood out prominently and were considerably enlarged and hyperemic. The spleen was from two to three times larger than normal. The lungs, heart, liver and other organs showed no distinctive gross pathologic changes. Microscopically, the tonsils and the lymphoid tissue of the mesentery showed characteristic simple "myeloid" hyperplasia. Microscopically, the spleen showed a diminution in the number and size of the lymphoid follicles. On first impression, the splenic pulp suggested marked hyperemia of the sinuses. On further examination, the exact structure of the splenic pulp had not been established. The microscopic changes in the bone-marrow had not yet been studied. The remaining viscera showed no pathologic features microscopically. An examination of the blood made before death showed: hemoglobin, 26 per cent (Dare); red blood cells, 1,750,000, and index, 0.7. The cells showed poikilocytosis and polychromatophilia, and erythroblasts were present. The white blood cells numbered 94,400, with a differential count of neutrophils, 51 per cent; lymphocytes, 36 per cent, and myelocytes 13 per cent.

There are several similar features in the two cases reported by Dr. Whitcher and in my case that came to autopsy. A striking feature is the peculiar pigmentation of the skin which at the time of autopsy suggested marked hemolysis. It is of interest to consider whether my case may not present the pathologic aspects of Dr. Whitcher's cases. All three of these cases appear to be characterized by primary diminution in the number of red cells, associated with the presence of immature red cells in the circulating blood, together with leukocytic changes suggesting those of leukemia. In my case, the myeloid hyperplasia of the tonsils and of the mesenteric nodes is of interest in relation to the possible leukemic changes. The histologic picture of the spleen suggests a possible deficiency of its hematopoietic function. This condition, therefore, would appear to be one of primary erythrocytogenic failure with associated myeloid hyperplasia. If the term "pseudoleukemia" is defined as a primary systemic lymphoid hyperplasia without an associated lymphocytic blood picture of leukemia, it could be ruled out in these cases, the predominant and apparently primary if not definitive change being an erythrocytogenic deficiency.

NICHOLAS M. ALTER: I have seen Dr. Whitcher's slides, which were stained by MacNeal's method. This produces marked contrast which does not give much possibility for mistake. The drawings do not do justice to it. The picture is striking, and it shows at times a more marked condition than in pernicious anemia.

B. R. WHITCHER: Some time after having seen these two or three children, a premature infant, a girl, was born at about 7 months in the Babies' Ward, and died at the age of 19 days. I made a blood count, and found more than 300 nucleated red cells to 100 leukocytes. In premature infants, born at 7 or 8 months, one does find a large number of these nucleated red cells, but rarely in an infant born at full term; the infants whose cases I reported were all born at full term.

HARRISON S. MARTLAND: How about the icteric index?

B. R. WHITCHER: I did not determine the icteric index. I had two cases in brothers, who had hemolytic icterus, and each had a splenectomy and made a good recovery; they are now normal. Before operation smears showed some nucleated red cells with some polychromatophilia, but not nearly as many nucleated red cells as those of the three cases reported.

A STUDY OF COMBINED CARBOHYDRATE IN THE BLOOD. ICHIRO KATAYAMA (by invitation).

The protein of the blood, which had been freed from sugar, when heated with second-normal hydrochloric acid in a bath of boiling water for two hours, yielded a substance giving the typical reduction of alkaline copper solutions. With phenylhydrazine, an ozazone was obtained which corresponded with the glucozazone. Combined sugar is present in greater amount in the plasma than in the corpuscles.

The blood was incubated for four hours at 40 C., with 0.3 per cent amylase solution and showed a marked increase in reducing substances with a much greater

increase in amino nitrogen. The increase in fermentable (true) sugar in blood after hydrolysis by amylase ranged from 9 to 29 mg. per hundred cubic centimeters of blood as dextrose.

The fact that the combined sugar cannot be demonstrated in the protein-free blood filtrate suggests that it is chemically combined with blood proteins and may be present in the blood as "gluco protein" — sugar combined with blood protein in a glucoside linkage.

The nonfermentable reducing substance in human blood averaged 25 mg. per hundred cubic centimeters as dextrose.

A Case of Paroxysmal Tachycardia in the Course of Active Subacute Bacterial Endocarditis. Arthur N. Foxe.

A man, aged 23, had an essentially unimportant history. The onset of the illness occurred two months before admission with a cold, cough and blood-streaked sputum. Several weeks later he was feverish and had night sweats and pain in the left loin and joints. Physical examination showed evidences of mitral stenosis. The general features led to a diagnosis of subacute bacterial endocarditis, which was confirmed by a positive blood culture for *Streptococcus viridans*. The subsequent course was febrile, with numerous peripheral, visceral and cranial embolic phenomena. Two weeks before death an attack of paroxysmal auricular tachycardia began, the pulse rate being 188. The attack lasted for six days. During the illness, the patient received doses of digitalis and occasional hypnotics. Death was due to cerebral complications.

Postmortem examination showed petechiae of the skin and conjunctivae, petechiae of the pleura, left hemohydrothorax and hydropericardium, both unusual in this disease. There were auricular and interauricular septal myocardial petechiae, mitral and aortic valvular and mural endocarditis, and infarctions of the spleen and kidneys. Microscopic examination showed myocardial petechiae, as observed, focal fibrotic myocardial change and one large focal accumulation of monothelial cells in the ventricular myocardium.

Cardiac arrhythmias are said to be rare in active subacute bacterial endocarditis. Bickel could find no case in the literature; he described a case of heart block. Rothschild, Sachs and Libman described the only other case that I have been able to find in the literature, that of a patient with auricular fibrillation. Myocardial involvement is often said to be rare in this disease. Blumen found active inflammatory changes in 1 per cent of the cases, Clawson in 24 per cent and Thayer in 61 per cent.

The discrepancies in the studies cited and the difference between apparent fact and the valid view of Mackenzie, that myocardial involvement is usually present when there is an active endocarditis, are strange. For a better clinical and pathologic understanding of the disease, it is essential that these opposing views be estimated correctly. Symptomatically, clinically and pathologically, there are barriers to the knowledge of the myocardial involvement in subacute bacterial endocarditis. "These patients," as Debré accurately stated, "are cachectics, never cardiacs." The anemia and the peripheral embolic phenomena are so striking and varied that they attract almost the entire attention of the clinician. This is fairly different from the monotonous regularity of the congestive phenomena of chronic cardiovalvular disease, in which the heart signs and close digitalis control become so engaging. Pathologically, the endocardial manifestations so obtrude that one largely neglects a more careful study of the myocardium. It is probable that when the studies of the living and dead myocardium in subacute bacterial endocarditis have been pursued with the persistence with which those of the presystolic murmur and the Aschoff body in acute rheumatic fever have been pursued, one will find little, if any, disparity in the frequency of myocardial involvement in the two diseases. The report of this case is a small effort in that field.

Book Reviews

DIE BIOLOGIE DER PERSON: EIN HANDBUCH DER ALLGEMEINEN UND SPIZIELLEN KONSTITUTIONSLEHRE UND MITARBEIT ZAHLREICHER FACHMÄNNER. Herausgegeben von PROF. DR. T. BRUGSCH und PROF. DR. F. H. LEWY. Parts 4-7. Berlin: Urban & Schwarzenberg, 1926 and 1927.

The first volume of this work was reviewed in the ARCHIVES (**4**:151, 1927). The fourth issue of the great system of the biology of the person brings an excellent contribution by E. Keeser on the relations between constitution and action of drugs. In collaboration with Joachimoglu the author has done pioneer work in this new field of constitutional and pharmacodynamic research which offers many possibilities for further investigations, especially with regard to idiosyncrasy. Not only does constitution influence the action of drugs, but also drugs when given for a long period of time influence the constitution. After a general discussion of the internal and external factors that determine the effect of a drug on a given individual, Keeser takes up the various chemical substances in detail. Though the discussion is brief, one will find many valuable suggestions especially in the paragraphs on alcohol, anesthetics, alkaloids, hypnotics and organic and inorganic poisons. A discussion of industrial poisonings is also given.

The next chapter is devoted to the question of constitution and the abuse of narcotics. Ernst Joël and Fritz Fraenkel try to cover this intricate problem in forty-two pages and succeed well, though some of their discussions are rather superficial. As far as prohibition is concerned, the authors come to the conclusion that its advantages by far excel its disadvantages.

Using statistical data as a basis, G. Florschuetz speaks of the great importance of the constitution in calculating the risk of life insurance.

Robert Heindl points out that a tremendous material for constitutional research has accumulated in the archives of the identification services of the various nations. This material has the great advantage that it is based on uniform and exact methods, and that it not only contains the measurements, photographs and fingerprints of millions of people, but also gives an account of the story of their life and of their mental and moral qualities. Heindl describes in detail the methods of anthropometry as applied for criminalistic purposes, and of the portrait parlé introduced by Bertillon. It is interesting to learn that some of the modern criminalistic methods were used already in ancient times, and that a warrant issued at Alexandria in 145 B.C. read very much up to date. The study of Heindl's paper can be highly recommended.

The greater part of the fifth issue is devoted to the factors which determine the person's fitness for life. Carl Coerper takes up the question from the somatic and W. Peters from the psychologic point of view. When the time comes that a young person has to choose his future occupation, not only his inclination but his constitution should be taken into consideration; if this is done, much disappointment and despair can be avoided. The physician well trained in the science of constitution should have a leading position among those who deal with problems of occupation.

In the same issue, Coerper also discusses constitution in relation to sport. Every person has an inclination for a certain kind of exercise which determines his favored sport. If sport is to be healthful, it should be adjusted to the person's constitution.

In issue six, Brugsch gives a complete presentation of the morphology of the person. He describes the different types as advanced especially by the French school and devotes much space to the methods of anthropometry, following the work of R. Martin. Mathematical measurements eliminate subjectivity in judgment, and uniformly applied not only make possible the study of the constitutional types

of a single population, but offers exact material for the comparison of the human races with one another.

The problem of inferiority is taken up by Max Berliner. Inferior forms of persons (Kuemmerformen) are those who do not reach the height of development which is characteristic of their race. The conception of inferior forms has been introduced by F. von Kraus and T. Brugsch and is apt to replace so-called infantilism. Inferiority, which in this connection is used merely from a morphologic standpoint, may affect the whole organism or parts of it. For clinical purposes, Berliner distinguishes the following forms of inferiority: (1) forms associated with diseases of the glands of internal secretion and with anomalies of the skeleton, to which group belong hypophysiary dwarfism, dyscerebral dwarfism, mongolism, cretinism, and myxedema, (2) forms with predominating changes of the skeleton, such as rickets, chondrodystrophia, osteopsathyrosis, enchondromas and Moeller-Barlow's disease, and (3) dystrophic forms.

The chapter on the individual development in infancy and childhood by A. Schlossmann and A. Eckstein is short and contains little of value.

The seventh issue contains, besides chapters on childhood, puberty and senility by M. Berliner and on the changing judgment concerning the artistic forms of the human body by Eugen Hollander, a contribution by Georg W. Schorr in which pathologists will be much interested. This contribution deals with the significance of thanatology for the biology of the person. Thanatology is the science of death. Those whose duty it is to explain the occurrence of death on the basis of anatomic observations often fail to explain why death has occurred at a certain moment and not hours or days before or afterward. Death does not result from a single cause, but is the outcome of a series of conditions which are incompatible with life. It is often impossible to determine all these conditions after life has vanished, and the work at the necropsy table necessarily has to be completed by studies immediately before and during death.

LABORATORY TECHNIQUE. THE METHODS EMPLOYED AT ST. LUKE'S HOSPITAL, NEW YORK. F. C. WOOD, KARL VOGEL and L. W. FAMULENER. Third edition, revised and enlarged. Price, $3.75. Pp. 318. New York: James T. Dougherty, 1929.

The first edition appeared in 1917. Since then a great increase has taken place in the demands on the clinical laboratory necessitating a thorough revision in order to reflect adequately present practice. There are three sections: histologic methods by F. C. Wood, 20 pages; clinical pathology and clinical analytic methods by Karl Vogel, 100 pages; and bacteriology including serology by L. W. Famulener, 184 pages. The book lays no claim to being a complete guide to all laboratory methods; Kahn's test for syphilis, for instance, is not mentioned. The methods described are those used in St. Luke's Hospital in New York, and "preference is given to methods that appear to be most reliable at the same time as they are practical." The book is an eminently safe technical guide so far as it goes and of interest to all workers in clinical laboratories as an illustration of the actual practice in a large hospital.

Books Received

PRINCIPLES OF PATHOLOGY FOR PRACTITIONERS AND STUDENTS. By H. D'Arcy Power, M.D., F.R.P.S., Professor of Pathology, College of Physicians and Surgeons, San Francisco, and William W. Hala, M.D., Assistant Professor of Pathology, Long Island College Hospital, Brooklyn, N. Y. Price, $10. Pp. 787, with 298 illustrations. New York: D. Appleton and Company, 1929.

DISEASES OF THE THYROID GLAND. By Arthur E. Hertzler, M.D., Surgeon to the Halstead Hospital. With a chapter on Hospital Management of Goiter Patients by Victor E. Chesky, M.D., Associate Surgeon to Halstead Hospital. Price, $7.50. Pp. 286. Second edition, entirely rewritten. St. Louis: C. V. Mosby Company, 1929.

ARBETEN FRÅN KAROLINSKA INSTITUTETS PATOLOGISKA AVDELNING UTGIVNA AV FOLKE HENSCHEN. Volume 4, 1928. Professor i patologisk anatomi. Stockholm, 1929.

A CONTRIBUTION TO THE KNOWLEDGE OF LYMPHOGRANULOMA INGUINALE. By Sven Hellerström, Lic. Med. From the Pathological Department (Head: Professor F. Henschen), and the Dermato-Venereological Clinic (Head: Professor J. Almkvist), of the Caroline Institute. Pp. 224, with 16 illustrations. Stockholm: P. A. Norstedt & Söner, 1929.

This is a complete and well illustrated presentation of the present knowledge concerning inguinale lymphogranuloma. Forty-seven carefully studied cases are reported. The bibliography contains 229 references. The main points in the author's summary will be given in abstracts from the current literature.

THE ORIGIN OF MALIGNANT TUMORS. By Theodor Boveri, University of Würzburg. Translated from German by Marcella Boveri, with a foreword by Maynard M. Metcalf, Johns Hopkins University. Cloth. Price, $2.50. Pp. 128. Baltimore: Williams & Wilkins Company, 1929.

REPORT OF THE LABORATORY AND MUSEUM OF COMPARATIVE PATHOLOGY OF THE ZOOLOGICAL SOCIETY OF PHILADELPHIA. By Herbert Fox, M.D., Pathologist. Pp. 65. 1929.

This report contains interesting notes on diseases of various kinds of animals.

DIE BIOLOGIE DER PERSON. Ein Handbuch der allgemeinen und speziellen Konstitutionslehre unter Mitarbeit zahlreicher Fachmänner. Volume 4. Von Prof. Dr. T. Brugsch und Prof. Dr. F. H. Lewy. Price, 13 marks. Pp. 825-986. Berlin: Urban and Schwarzenberg, 1929.

THE FORTY-FOURTH ANNUAL MEDICAL REPORT OF THE TRUDEAU SANATORIUM AND THE TWENTY-FOURTH MEDICAL SUPPLEMENT FOR THE YEAR ENDING SEPTEMBER 30, 1928. Together with the Twelfth Collection of the Studies of the Edward L. Trudeau Foundation for Research and Teaching in Tuberculosis. Price, 50 cents. Pp. 257. Trudeau, N. Y., 1928.

INDEX TO VOLUME 7

The star (*) preceding the page number indicates an original article. No author entries are made except for original articles and society transactions. Book Reviews, Obituaries and Society Transactions are indexed under these headings in their alphabetical order under the letters B, O and S, respectively.

INDEX TO VOLUME 7

INDEX TO VOLUME 7